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LONG-TERM PAIRED ASSOCIATIVE STIMULATION FOR RESTORATION OF MOTOR FUNCTION AFTER SPINAL CORD INJURY

Andrei Rodionov

ACADEMIC DISSERTATION

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To my family

ABSTRACT

Spinal cord injury (SCI) is a devastating condition and consequent loss of motor control remains one of the main causes of disability. Motor recovery after SCI depends on the amount of spared and restored neural connections in the spinal cord. Most SCIs are incomplete and even neurologically complete injuries possess some spared neural connections. Damaged motor pathways can be reactivated by external stimulation. However, current treatment approaches are mainly palliative, such as assisting adaptation to impairments. Thus, there is a need for novel therapies to induce neuroplasticity in the spinal cord and strengthen weak and disrupted neural connections.

In this thesis, paired associative stimulation (PAS) was applied as a long-term treatment for chronic incomplete SCI of traumatic origin. PAS is a non-invasive neuromodulation paradigm where descending volleys induced by transcranial magnetic stimulation (TMS) of the motor cortex are timed to coincide with antidromic volleys elicited by peripheral nerve electrical stimulation (PNS). The stimulation protocol was designed to coincide TMS- and PNS-induced volleys at the cortico-motoneuronal synapses in the spinal cord. Continuous pairing of TMS and PNS stimuli can change synaptic efficacy and produce long-term potentiation (LTP)-like plasticity in the corticospinal tract. Augmentation of synaptic strength at the spinal level has clear therapeutic value for SCI, as it can enhance motor control over paralyzed muscles.

The aim of the thesis was to investigate the possible therapeutic effects of long-term PAS on hand and leg motor function in individuals with chronic incomplete SCI of traumatic origin.

Study I explored long-term PAS therapeutic potential by providing long-term PAS until full recovery of hand muscle strength or until improvements ceased. The PAS protocol was designed to coincide TMS- and PNS-induced volleys in the cervical spinal cord, which is both the location of the stimulated lower motor neuron cell bodies and the site of the injury. Improvements up to normal values of hand muscle strength (Manual Muscle Test [MMT]) and increased amplitude of motor evoked potentials (MEPs) were obtained after more than 1-year stimulation in a participant with SCI. The participant regained almost complete self-care of the upper body. This was the first demonstration of restoring normal strength and range of movement of individual hand muscles by means of long-term PAS. The effect persisted over 6 months of follow up.

Study II probed the effects of long-term PAS on leg muscle strength and walking in a group of five people with SCI. The PAS protocol was designed to coincide TMS- and PNS-induced volleys in the lumbar spinal cord but the site

of the injury was in the cervical spinal cord. Long-term PAS delivered for 2 months significantly increased the total lower limb MMT score. This effect was stable over a 1-month follow up. Walking speed increased after 2 months of PAS in all participants. This study was the first demonstration that long-term PAS may significantly increase leg muscle strength and affect walking. The MMT score prior to the intervention was a good predictor of changes in walking speed.

Study III developed a novel technique that enables probing neural excitability at the cervical spinal level by utilizing focal magnetic coil and anatomy-specific models for re-positioning of the coil. The technique enabled recording of highly reproducible MEPs and was suitable for accurate maintenance and retrieval of the focal coil position at the cervical level.

In summary, this thesis contributes to the understanding of therapeutic efficacy of long-term PAS for restoration of motor control over hand and leg muscles after chronic SCI. This work challenges the view that chronic SCI is an irreversible pathologic condition and demonstrates the possibility of restoring neurological function many years postinjury when spontaneous recovery is extremely rare. The increased amplitude of MEPs, sustainable motor improvements, and the effects observed regardless of injury location indicate that PAS induces stable changes in the corticospinal pathways.

TIIVISTELMA

Selkäydinvamma on ihmiseen kokonaisvaltaisesti vaikuttava tila, ja motorinen heikkous on yksi tärkeimmistä tekijöistä, jotka aiheuttavat rajoituksia päivittäiseen elämään. - Nykyiset hoitomenetelmät pääasiassa lievittävät oireita. Ne helpottavat kivun ja spastisuuden hallintaa ja sopeutumista vammaan sekä estävät sekundaarisia komplikaatioita. Keskushermosto voi kuitenkin järjestyä uudelleen sopeutuakseen heikentyneeseen toimintaan, ja tätä muovautuvuutta voidaan käyttää terapeuttisena mahdollisuutena. Vaurioituneet hermoradat voidaan aktivoida uudelleen ulkoisella stimulaatiolla. Toipuminen selkäydinvamman jälkeen riippuu niistä selkäytimen hermoyhteyksistä, jotka ovat säästyneet ja jotka on onnistuttu palauttamaan. Usein selkäydinvammat ovat osittaisia, ja neurologisesti täydellisissäkin vammoissa on joitakin säästyneitä hermoyhteyksiä. Uusilla hoitomenetelmillä voidaan aktivoida selkäytimen neuroplastisuutta ja vahvistaa heikkoja ja katkenneita hermoyhteyksiä.

Tässä väitöskirjassa kaksoisstimulaatiota (PAS) käytettiin pitkäaikaisena hoitona potilailla, joilla oli krooninen, traumaattinen osittainen selkäydinvamma. PAS on neuromodulaatiomenetelmä, jossa aivokuoren transkraniaalinen magneettistimulaatio (TMS) synkronoidaan perifeeristen hermojen sähköstimulaatioon (PNS). Stimulaatioprotokolla suunniteltiin niin että TMS: n ja PNS: n synnyttämät aktivaatiot kohtaavat selkäytimen synapseissa. Jatkuva TMS:n ja PNS:n aikaansaamien ärsykkeiden kohtaaminen selkäydintasolla voi voimistaa synapsien tehokkuutta ja tuottaa pitkäaikaisen synaptisen potentiaation (long-term potentiation, LTP) selkäytimessä. Synaptisen tehokkuuden kasvu selkäytimessä todennäköisesti parantaa lihasten tahdonalaista hallintaa.

Väitöskirjan päätavoitteena on ollut tutkia pitkäaikaisen kaksoisstimulaation (PAS) mahdollisia terapeuttisia vaikutuksia käden ja jalkojen tahdonalaiseen lihasaktiivisuuteen henkilöillä, joilla on traumaattinen krooninen osittainen selkäydinvamma.

Tutkimuksessa I selvitin pitkäaikaista PASin terapeuttista potentiaalia antamalla PAS-hoitoa niin kauan kunnes käden lihasten voima palautui kokonaan, tai voimassa ei tapahtunut enää kasvua. Yli vuoden kestäneen stimulaation jälkeen käsien lihasvoimat kohenivat normaaliarvoihin (Manuaalinen lihas testi, MMT) osallistujalla, jolla oli krooninen osittainen neliraaajahalvaus. Sen lisäksi herätevastet (motor-evoked potentials) kasvoivat. Koehenkilön ylävartalon lihashallinta palautui lähes täydellisesti. Tämä on ensimmäinen osoitus yksittäisten käsilihasnormaalin voiman ja liikeratojen palautumisesta pitkäaikaisen PAS:n avulla selkäydinvammapotilaalla. Vaikutus säilyi 6 kuukauden seurannassa.

Tutkimuksessa II tutkittiin pitkäaikaisen PAS: n vaikutuksia alaraajalihasten voimaan ja kävelyyn viidellä henkilöllä, joilla on krooninen tetraplegia. Kahden kuukauden ajan annettu pitkäaikainen PAS lisäsi merkittävästi alaraajojen MMT-pistemäärää keskimäärin yhdellä pisteellä lihasta kohden. Tämä tulos säilyi kuukauden seurannassa. Kaikkien osallistujien kävelynopeus kasvoi PAS-hoitojakson jälkeen. Tutkimus on ensimmäinen osoitus siitä, että pitkäaikainen PAS voi lisätä merkittävästi alaraajojen lihasvoimaa. MMT-pistemäärä ennen interventiota ennusti hyvin kävelynopeuden muutoksia.

Tutkimuksessa III kehitettiin uusi tekniikka, joka mahdollistaa magneettistimulaation selkäydinalueella käyttäen fokaalista magneettikelaajaa ja pään anatomisia malleja magneettikelan toistettuun kohdentamiseen. Menetelmä mahdollisti toistettavien MEP-signaalien mittaamisen sekä kelan sijainnin tarkan, toistettavan paikannuksen ja kohdentamisen niskan alueella.

Yhteenvetona voidaan todeta, että väitöskirja lisää ymmärrystä pitkäaikaisen PAS: n terapeuttisesta tehosta ylä- ja alaraajalihasten hallinnan palauttamisessa ja omatoimisuuden lisäämisessä kroonisen selkäydinvamman jälkeen. Väitöskirja haastaa käsityksen kroonisen selkäydinvamman aiheuttamien toimintahäiriöiden pysyvästä luonteesta. Sen lisäksi väitöskirja osoittaa mahdollisuuden palauttaa lihasaktiivisuutta nimenomaan kroonisessa selkäydinvammassa, jossa spontaani koheneminen on erittäin harvinaista. Voimistuneet lihasvasteet ja pysyvä lihashallinnan parannus vamman sijainnista riippumatta osoittavat, että PAS oikein käytettynä muokkaa liikejärjestelmää hyödyllisellä tavalla.

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LIST OF ORIGINAL PUBLICATIONS

This doctoral thesis consists of a summary and the following original studies, which are referred to in the text by their roman numerals:

- I **Rodionov A**, Savolainen S, Kirveskari E, Mäkelä J.P, Shulga A.
Restoration of hand function with long-term paired associative stimulation after chronic incomplete tetraplegia: a case study. *Spinal Cord Series and Cases* 5, 81, 2019
- II **Rodionov A**, Savolainen S, Kirveskari E, Mäkelä J.P, Shulga A.
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- III **Rodionov A**, Tolmacheva A, Kirveskari E, Mäkelä J.P, Shulga A.
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AUTHOR'S CONTRIBUTION

Study I: Restoration of hand function with long-term paired associative stimulation after chronic incomplete tetraplegia: a case study

The author performed the stimulations and collected part of the data independently and together with the third and the last author. The author was responsible for data processing and analysis and wrote the first version of the manuscript. The author significantly contributed to manuscript editing and preparation of the final version of the manuscript.

Study II: Effects of long-term paired associative stimulation on strength of leg muscles and walking in chronic tetraplegia: a proof-of-concept pilot study

The author performed stimulations of patients 2 to 5. The author performed the mapping of the motor cortex together with the last author and neurophysiological measurements together with the third author. The author participated in data collection and analysis. The author wrote the first version of the manuscript and contributed to manuscript editing and preparation of the final version of the manuscript.

Study III: The use of electronic coil location control for focal magnetic stimulation at the cervical level

The author pointed out the methodological necessity to the use of coil tracking system and contributed to the design of the study. The author collected the data together with the second and the last author and performed the analysis together with other authors. The author wrote the first version of the manuscript and was responsible for manuscript editing and preparations of the final version of the manuscript.

ABBREVIATIONS

ADM	abductor digiti minimi
AIS	ASIA impairment scale
ANOVA	analysis of variance
AP	action potential
APB	abductor pollicis brevis
ASIA	American Spinal Cord Injury Association
AT	appearance threshold
BB	Box and Block test
BR	brachioradialis
CMAP	compound motor action potential
CMCT	central motor conduction time
CMEP	cervico-medullary motor evoked potential
CNS	central nervous system
CSP	cortical silent period
CST	corticospinal tract
eEFM	estimated electric field maximum
EEG	electroencephalography
EF	electric field
e.g.	exempli gratia
EMG	electromyography
EPSP	excitatory postsynaptic potentials
etc.	et cetera
FDI	first dorsal interosseous
ICC	intra-class correlation coefficient
ICF	intracortical facilitation
ISI	interstimulus interval
LEMS	lower extremity motor score
LMN	lower motor neuron
LTP/D	long-term potentiation/depression
M1	primary motor cortex
MAS	Modified Ashworth Scale
MEP	motor evoked potential
MMT	manual muscle test
MN	motor neuron
MRI	magnetic resonance imaging
MSO	maximum stimulator output
MT	motor training
MU	motor unit
NBS	navigated brain stimulation
NHPT	Nine Hole Peg Test
NLI	neurological level of injury

NMDA	N-methyl-D-aspartate
nTMS	navigated transcranial magnetic stimulation
PAS	paired associative stimulation
PMC	premotor cortex
PMCT	peripheral motor conduction time
PNS	peripheral nerve stimulation
RMT	resting motor threshold
ROM	range of motion
rTMS	repetitive transcranial stimulation
S1	primary somatosensory cortex
SAI	short afferent inhibition
SAS	spinal associative stimulation
SCI	spinal cord injury
SCIM	Spinal Cord Independence Measure
SICI	short-interval intracortical inhibition
SMA	supplementary motor area
SSEP	somato-sensory evoked potential
STDP	spike-timing dependent plasticity
TMS	transcranial magnetic stimulation
UEMS	upper extremity motor score
UMN	upper motor neuron
WHO	World Health Organization

1 INTRODUCTION

Spinal cord injury (SCI) is a devastating condition that disconnects the brain from the rest of the body. Initial damage to the spinal cord is followed by a cascade of secondary pathological processes that lead to various dysfunctions [1], [2]. Loss of voluntary motor control over limb muscles is a common clinical manifestation of SCI, which has been historically understood as totally irreversible as described e.g. in the Edwin Smith papyrus [3] as a condition “...that cannot be healed”. On the basis of current knowledge, most SCI cases are incomplete [4], which means that some amount of neural fibers within the motor and sensory tracts to the limbs remain intact and even clinically complete SCIs may still have a few spared axons [5]. Despite this knowledge and progress in early surgical management of acute SCI [6], rehabilitative strategies for chronic injury are still mainly palliative and recovery at the chronic stage is extremely rare [7]. Truly curative approaches are therefore needed.

Spared weak and inactive spinal connections can be restored. Evidence from basic research [8]–[10] accumulated during the last two decades suggests that neural regeneration is slow but possible. An appropriately selected approach could reactivate inactive connections within the spinal cord and return signal transmission between the brain and the rest of the body, fostering recovery of voluntary motor control over paralyzed limbs. Nowadays, selective stimulation of different targets within the CNS is available for clinical use. For instance, with state-of-the-art navigated transcranial magnetic stimulation (nTMS) [11], it is possible to noninvasively modulate excitability of descending motor pathways. A combination of this method with peripheral nerve stimulation (PNS), called paired associative stimulation (PAS) [12], can induce durable changes in the motor cortex and the corticospinal tract.

This thesis is based on the novel PAS protocol [13], [14] developed in the BioMag Laboratory at the Helsinki University Hospital. This protocol is designed to strengthen weak and inactive spinal connections. In the following chapters, the background and methodology of PAS and the results of the administration of multiple PAS sessions over many months in chronic tetraplegia and development of a method for studying neural excitability at the spinal cord level will be summarized and critically discussed.

2 BACKGROUND

2.1 HUMAN SENSORIMOTOR SYSTEM

2.1.1 GENERAL PRINCIPLES OF ORGANIZATION

Motor control is maintained by a hierarchical system regulating voluntary movements, balance, coordination, and reflexes [15]. The system generates complex signal patterns traveling from the cortex to muscles via the motor pathways and receives sensory information flowing to the cortex through the sensory pathways. The system is based on closed-loop mechanisms utilizing sensory feedback to guide motor behavior [16], [17]. Although these mechanisms are spatially distributed, they act in synchrony to provide a robust neural background for motor control. Thus, the function of an injured element can be partially compensated by other structures [18], which undergo anatomical [19] and functional reorganization [20] to foster recovery [21]. Knowledge about the serial and parallel organization of motor control is important for understanding the pathophysiology of SCI and for development of accurately targeted therapeutic neuromodulation [16], [22].

The commonly accepted gross anatomical division of the human motor system consists of 1) the motor cortex, which includes the primary motor cortex (M1), the premotor cortex (PMC), and the supplementary motor area (SMA); 2) the spinal cord consisting of descending motor pathways and spinal neural circuits; and 3) peripheral nerves [15], [18]. Subcortical supraspinal structures, such as the cerebellum, the basal ganglia, and various nuclei are also involved in motor control. The functional hierarchy of the motor system defines which tasks are executed at each level. The motor cortex regulates complex movements and sequences of movements and plans motor behavior by evaluating sensory feedback [23]. Subcortical supraspinal structures perform high-order control of muscle tone, posture, and spinal reflexes [18]. The spinal cord transmits neural commands from the brain to the rest of the body and executes spinal-level control of somatosensory, nociceptive, autonomic, and motor functions [15], including simple reflexes [24] and central pattern generators [25]. Finally, peripheral nerves comprise what Sir Charles Sherrington called “the final common pathway” [26], or motor neurons (MN) where convergence of all motor commands occurs for transmission to separate muscles and muscle groups.

2.1.2 CEREBRAL CORTEX

The cytoarchitecture of the primary motor cortex is characterized by pyramidal neurons, which are the largest cells in the central nervous system (CNS) [27]. Pyramidal neurons are upper MNs that direct their processes to the spinal cord within the corticospinal tract, where they have synaptic contacts with alpha motoneurons (lower MNs) [28]. Pyramidal neurons of the primary motor cortex (M1) make up approximately 40% to 50% of the corticospinal fibers. The remaining fibers originate mainly from the PMC and SMA [18]. All these cortical regions also project to the brainstem at the origin of the reticulospinal tract, which is an indirect route to the spinal cord [18]. The motor cortex is the origin of the pyramidal tract [17].

The M1, PMC, and SMA have cortico-cortical connections. The motor cortex is also connected with the primary somatosensory cortex (S1) and to the posterior parietal cortex [15], forming a distributed network controlling movements. The M1 primarily contributes to fine hand movements, skilled locomotion that requires continuous visuomotor feedback [17], whereas the PMC selects motor programs and plans voluntary movements, including the preparation for movement based on sensory input or on internal representations [29]. The SMA is involved in programming complex sequences of movements [30] and coordinating bilateral movements [31].

Somatotopy [32] refers to spatial presentations of different body areas within the M1 and S1 cortical strips. The motor cortices of the left and right hemispheres are connected via the corpus callosum, which transmits interhemispheric facilitatory [33] and inhibitory influences [34]. The motor cortex receives sensory feedback from receptors in muscles, tendons, joints, and skin relayed via ascending pathways and thalamus to S1 [17], [32]. Area S1 provides the main activating input to the motor cortex, integrating motor and sensory systems together. Sensorimotor integration, somatotopy, and interhemispheric connections form the basis of functional organization of the motor system and are widely used in neuromodulation [35]–[37].

2.1.3 SPINAL CORD

The spinal cord is a segmentally organized structure inside the spinal canal of the vertebral column [38]. It is grossly divided into the following four levels: cervical (8 segments, C1-C8), thoracic (12 segments, T1-T12), lumbar (5 segments, L1-L5), and sacral level (5 segments, S1-S5). The spinal cord originates in the brainstem and terminates in the conus medullaris at the level of the L1 and L2 vertebrae. The spinal cord comprises the butterfly-shaped grey matter (constituting neuron cell bodies) and surrounding white matter (with myelinated and unmyelinated fibers), including axons of upper MNs [28]. The main grey matter areas are called the dorsal and ventral horns. The main white matter areas are the dorsal and ventral columns. The cytoarchitecture of the spinal cord is characterized by the presence of 1) efferent neurons (alpha motor neurons and gamma motor neurons), 2) afferent projection neurons, and 3) interneurons [38].

The corticospinal tract (CST) is the main descending (pyramidal) pathway in the spinal white matter that carries information associated with voluntary movement of arms and legs [39]. The CST originates from the motor cortex and splits into two tracts. At the pyramidal decussation, the vast majority the fibers cross over to the contralateral side, forming the lateral CST (Figure 1). When these fibers reach the ventral horn of their terminal spinal segment, they form synaptic contacts either directly to alpha MNs or to interneurons. The remaining axons continue ipsilaterally as the anterior CST and cross over to the contralateral side at the segmental level and synapse on alpha MNs or interneurons in the anterior horn. Thus, the lateral CST consists of direct monosynaptic pathways for motor commands [40]. The rubrospinal tract provides an indirect alternative pathway for voluntary motor inputs. Other descending tracts originating in the lower brainstem [17] are located in the medial spinal cord. These tracts belong to the extrapyramidal descending system, which mediates balance and postural adjusting movements. Ascending tracts carry sensory information from the body to the brain and include the dorsal column-medial lemniscal pathway, subdivided into the cuneate fasciculus (sensory input from the upper extremities) and the gracile fasciculus (sensory input from the lower extremities), the anterolateral system (pain and temperature), and the somatosensory pathways to the cerebellum (unconscious proprioception) [38].

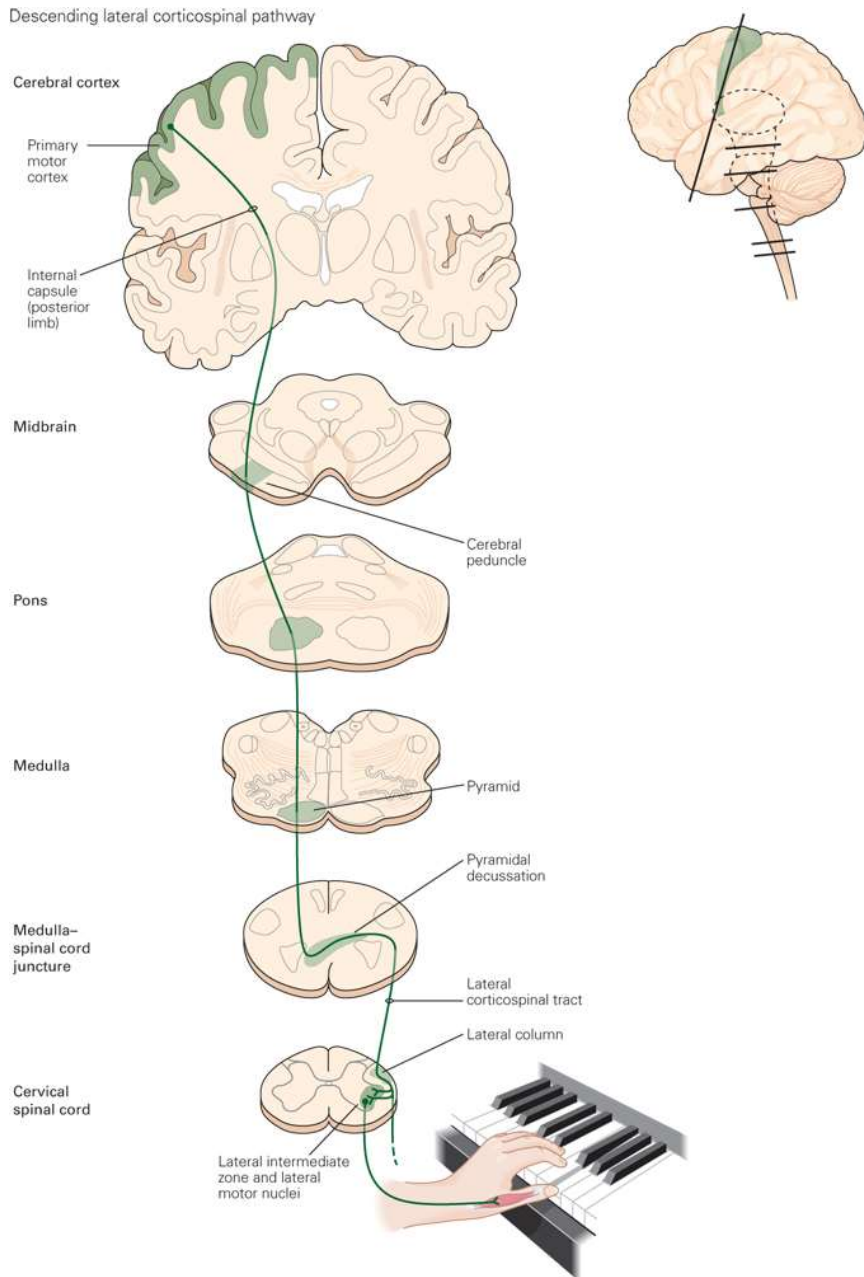


Figure 1 The lateral corticospinal pathway. Figure reprinted from Principles of Neural Science, ed. Kandel et al, Fifth edition, 2013 with permission from McGraw-Hill Education.

2.1.4 PERIPHERAL NERVES

Peripheral nerves originate from the left and right side of each spinal segment, first as a fascicle of spinal rootlets, which form a spinal root. The cervical and upper thoracic rootlets are directed caudally at an acute angle to the spinal cord and have a short upwardly directed segment when they pass through the intervertebral foramen [41]. The C5-C8 cervical roots and first thoracic root T1 join to form the brachial plexus [42], which gives rise to the musculocutaneous, axillary, radial, median, and ulnar nerves that innervate the shoulder, arm, forearm, and hand. The spinal roots that innervate the lower limbs form the lumbosacral plexus (L1-S4) [43], which gives rise to the gluteal, femoral, obturator, tibial, and common peroneal nerves.

Peripheral nerves consist of axons of alpha motor neurons (lower MNs) [28], which innervate extrafusal fibers of the skeletal muscles and regulate their power. Gamma motor neurons innervate intrafusal fibers and detect the change in the muscle length to monitor stretch [28]. One alpha MN can innervate several muscle fibers. A motor unit (MU) consists of an individual alpha MN and all muscle fibers that it innervates. All alpha MNs innervating a single muscle are clustered together and called a MN pool [28]. The force produced by a muscle during a voluntary contraction depends on the number of recruited MUs and the rates of action potentials [44].

Most peripheral nerves are mixed nerves consisting of both motor and sensory fibers. The axon of a primary sensory neuron, whose cell body is located in the dorsal root ganglia (with some exceptions) [45], enters the spinal cord to synapse directly to a second-order neuron or interneuron [45]. Sensory input modulates the activity of motor neurons at the spinal level via the simple reflex arc and influences supraspinal centers, including various nuclei, the reticular formation, and the somatosensory cortex.

2.2 SPINAL CORD INJURY (SCI)

2.2.1 CLASSIFICATION, EPIDEMIOLOGY, PROGNOSIS AND REHABILITATION

SCI is a devastating condition that leads to a range of disabilities, including motor and sensory deficits, and dramatic disturbances of physiological processes, mental health [46], and social life [4]. Disruption of neural connections to supraspinal centers causes multiple neurological problems, such as difficulties with respiration, bowel and bladder dysfunction, spasticity, neuropathic pain, and autonomic dysregulation [1], [2]. The risk of premature death is 2 to 5 times higher in people with SCI [47]. SCI often leads to social

isolation, which negatively affects mental and physical health. Mortality rates due to suicide among SCI patients is 3 times higher than in the general population [48].

SCI can be complete or incomplete. Sensory and motor function in the lowest sacral segment is absent in complete SCI. Most SCIs (up to 80%) are incomplete [4] and even complete SCIs possess some spared neural connections across the lesion [5]. SCIs are classified (in descending order of injury severity) from grade A to grade E. Grade A refers to complete SCI, B to sensory incomplete, C to motor incomplete (less than half of key muscles below the single neurological level of injury [NLI] possesses a muscle grade ≥ 3 corresponding to active movement and full range of motion [ROM] against gravity), and D to motor incomplete SCI (at least half or more of key muscles below the single NLI has a muscle grade ≥ 3). E corresponds to normal motor and sensory functions [49]. SCI can be divided into tetraplegia and paraplegia. Tetraplegia is an impairment or total loss of motor or sensory function (or both) below the cervical segments in the arms, trunk, legs, and pelvic organs. Paraplegia is impairment or loss of functions only in the thoracic, lumbar, or sacral spinal segments [50].

SCI is classified by its cause. Traumatic SCI results from a traumatic event [51], whereas non-traumatic SCI can be caused e.g., by tumors or infection [2]. Historically, traumatic SCIs made up to 90% of the whole SCI population [51]. More recent reviews report a higher incidence of non-traumatic than traumatic SCI in some countries [2]. Finally, SCI is divided into acute and chronic stages by the timing of pathological events. Primary immediate stage (≤ 2 hours), early acute stage (≤ 48 hours), secondary subacute stage (≤ 14 days), intermediate stage (≤ 6 months), and chronic stage (≥ 6 months) can be identified [52]. Spontaneous recovery is important for restoration of motor and sensory function and can occur during the first 6 to 9 months after SCI and plateaus after 12 months [7]. Spontaneous recovery is rare after this time [53]. However, neurological function can be restored to a certain extent even at the chronic stage by means of clinical interventions [54], [55].

Classification of SCI is performed by employing the American Spinal Injury Association (ASIA) Impairment Scale (AIS) [50]. AIS is a reliable common measure used for diagnostic purposes worldwide [56]. It includes determination of NLI (cervical, thoracic, or lumbar) and examination of the preserved motor and sensory functions. NLI represents the most caudal segment of the spinal cord with intact sensation and muscle strength enabling movement against gravity [50]. Motor examination is performed by testing muscle functions corresponding to 10 myotomes to provide upper and lower extremity motor scores (UEMS and LEMS, respectively) and total motor score for each limb. Examination of sensory function consists of sharp-dull discrimination in the 28 dermatomes and generates pin prick and light touch sensory scores. Age, neurological level, and results of 3-day examination [57],

together with many other parameters [58], form the background for prognosis. In addition to a standard neurological examination, magnetic resonance imaging (MRI) is employed for evaluation of sites of contusion and white and grey matter damage [59].

According to The World Health Organization (WHO) report [51], the global incidence of SCI is 40 to 80 cases per million persons. The risk of SCI is two times higher for men [47] and higher for young adults and adults older than 60 years [1]. The main causes of traumatic SCI are traffic accidents, falls, and violence [47]. The incidence of traumatic SCI is increasing in many countries [60] despite preventive measures. The incidence of SCI in North America is approximately 39 cases per million and in Western Europe approximately 15 per million [61]. The SCI incidence in Sweden was 19.0 per million (2014–2015) [62], 10.2 per million in Denmark (1990–2012) [63], and 15.9 per million (2014) in Norway [64]. In Finland, the incidence in 2012 to 2013 varied between 25.1 and 38.1 per million [65].

SCI is currently considered as an irreversible disorder and no therapy that recovers normal body functions is available. Thus, supportive treatments and adaptation to impairments following injury are common rehabilitation strategies [66]. Consequently, rehabilitation and disability after SCI produce a substantial financial burden [57], [67]. Rehabilitation after SCI is an actively evolving field of medicine [55]. Many treatment options have been developed [68] but none can be considered as a universal cure. Surgical procedures (e.g., early decompression [6]) aim to place the spinal cord and nerves in optimal surroundings for recovery. Pharmacological treatment is another rapidly developing area [57], [69]. Growth-promoting factors, together with stem-cell [70] or Schwann-cell [71] transplantation and long-distance regeneration of neural fibers [72] are promising areas of study [73]. However, translation of the results into clinically feasible repair interventions remains a long-term goal [74]. Body weight support and locomotor training are widespread approaches improving balance, walking speed, and endurance [75] but have limited effectiveness in incomplete SCI [76]. Finally, implanted stimulators, robotic devices [77], exoskeletons [78], and brain-computer interfaces (BCI) [79] are emerging technologies that have some efficacy in restoration of upper extremity functions [80] and walking [81].

2.2.2 TRAUMA MECHANISMS, PATHOPHYSIOLOGY, AND NEURAL REORGANIZATION AFTER SCI

Knowledge on the trauma mechanisms and subsequent pathophysiological processes has been rapidly growing over recent decades with progress in cellular and animal research [1] and in neuroimaging [59] and neuroscience [8], [82]. Several key research findings have had an impact on the

development of therapeutic interventions. First, mechanical trauma rarely leads to total disruption of the spinal pathways [83]. Even if SCI is diagnosed as complete there may still be some axonal connections across the lesion [5]. This opens fruitful opportunities for neurorehabilitation via strengthening of spared neural connections. In addition, studies of injury mechanisms have determined optimal time windows for the most effective therapy [84]. Finally, cortical reorganization can contribute to various symptoms of SCI, e.g. neuropathic pain [85] and altered sensation [8]. This reorganization may be a potential target for treatment [86] and should be considered when SCI interventions are planned.

SCI is a dynamic pathological process. The primary injury is the initial mechanical damage of the spinal cord produced by impact of sharp or blunt force with transient or persistent compression, distraction or laceration, and transection of neural fibers by dislocated vertebrae or external objects [1], [87]. The primary injury is immediately followed by a cascade of pathological events that continue for several months, worsening the symptoms. These events are combined into a general concept of secondary injury [1], [88]. Vascular damage and the blood-spinal cord barrier destruction enlarge the lesion area. Subsequently, edema is accompanied by toxic accumulation of neurotransmitters, ionic imbalance, free radical formation, calcium influx, lipid peroxidation, and cell death [84]. Widespread pathological cellular reorganization includes axonal demyelination, apoptosis, degeneration, and glial reactivity, which leads to formation of a glial scar [5]. The glial scar progressively matures from days to years postinjury together with the formation of a cystic cavity [1]. In addition, injury is accompanied by a strong immune response [52].

SCI causes morphological and physiological changes that represent pathological reorganization [8] or pathological neuroplasticity [86]. Changes in cortical and corticospinal activity can occur immediately after the trauma and evolve rapidly [89]. Spontaneous electroencephalography (EEG) activity becomes slower after SCI [90] and probably reflects the first pathological response to deafferentation. EEG spectral reactivity is reduced and somatosensory evoked potentials (SSEPs) can be delayed or abolished [16]. The number of active corticospinal neurons can be reduced after SCI, as shown by increased resting motor threshold (RMT) [91]. Corticospinal excitability can be decreased as indicated by active motor threshold (AMT) [92] and the cortical silent period (CSP) [92] measurements. However, a decrease in activity of inhibitory circuits [93] was also revealed. In addition, latencies of MEPs induced by transcranial magnetic stimulation (TMS) can be prolonged [91], probably due to destruction of the rapidly conducting tract fibers [94]. However, it is still unclear how interactions between excitatory and inhibitory circuits [93], [95] at the cortical, subcortical, or spinal level contribute to

disability after SCI. Understanding these interactions can improve the diagnostic value of existing methods and lead to new effective SCI treatments.

In contrast to functional reorganization, atrophy of the spinal cord [96] and atrophy of the cortex [19] and reorganization of body representations in the motor and sensory cortices are relatively slow pathological processes [8], [97]. A decrease of regional white matter volume in pyramidal tracts [98] and cortical grey matter atrophy and reduced cortical thickness in the regions supplying paralyzed muscles have been reported [99]. These changes may be associated with decreased cortical connectivity or retrograde degeneration [8]. Moreover, deafferentation causes widespread changes in somatotopically organized brain regions [8], expressed as expansion of the cortical representations of intact body parts to the deafferented regions [100], [101] and shift of cortical activity to abnormal locations [20]. The degree of reorganization is inconsistent among different SCI subpopulations and can be influenced by many factors [20].

2.2.3 NEUROPLASTICITY AND MOTOR RECOVERY

After the injury, the CNS reorganizes itself to adapt to impaired function via plasticity of the residual neural connections. Many motor neurons can survive after SCI [9]; injured axons retain the ability to regenerate [9] and respond to synaptic inputs [8]. Collateral axonal sprouting and synaptic strengthening are thought to be the basis of neural reintegration [8], [10]. Adaptive neuroplasticity can be augmented by therapeutic stimulation. Therefore, it is important to understand the complex relationship between cellular mechanisms of neural regeneration, electrophysiological readouts, and motor gains following SCI.

Spared corticospinal connections account for motor recovery [102], [103]. There is robust evidence on the relationship between severity of SCI and spontaneous recovery of motor function during the first year after injury [104], [105]. Only 4% to 25% of individuals with complete SCI (AIS A) convert to incomplete AIS B or C. AIS B to AIS C conversion is seen in 15% to 40% of cases, AIS C to AIS D in 60% to 80% of cases, and AIS D patients improve in 95% of cases. Ambulation recovery follows the same trend. Approximately 14% of patients initially diagnosed with AIS A will ambulate; the corresponding percentage is 33% in patients with AIS B, 75% in AIS C, and about 100% in AIS D [106]. The quality of ambulation can vary across individuals; this includes independent ambulators (ability to walk independently, with or without braces and orthoses for <10 m) or those who require assistance. However, spontaneous recovery is limited and occurs during the first 3 months and usually plateaus by 9 months postinjury [1].

A combination of clinical and electrophysiological recordings can be useful for prediction of recovery of upper and lower limb functions [94]. In individuals with cervical SCI, the MEP amplitudes and the UEMS are highly correlated [107]. About 90% of participants with an absence of MEPs from the upper limbs did not regain active hand function [94]. An increase of MEP amplitudes over 12 months postinjury is associated with improvement of LEMS and walking [108]. However, MEPs recorded from leg muscles were not changed after locomotor training in spastic patients [109]. Thus, electrophysiological readouts and results of clinical examinations can be affected by many factors and represent different views on the process of recovery.

2.3 NON-INVASIVE NEUROMODULATION

2.3.1 *TRANSCRANIAL MAGNETIC STIMULATION AND MOTOR EVOKED POTENTIALS*

Transcranial magnetic stimulation (TMS) is a non-invasive method to artificially activate neurons in the brain by strong magnetic field gradients [110], [111]. The first TMS instrumentation suitable for activation of the primary motor cortex (M1) and recording TMS-evoked motor responses was introduced in 1985 by Barker and colleagues [112]. TMS does not cause discomfort or pain usually elicited by transcranial electrical stimulation [113]. A loaded capacitor, a switch, and a magnetic coil are required for TMS. Once the switch is closed, a brief high-amplitude electric current flows through the coil and generates a strong (approximately 1-2 Tesla) rapidly changing (approximately 100-200 microseconds) magnetic field. The magnetic field penetrates the scalp and the skull and induces an electric field (approximately 100-200 Volt/meter) in the conductive tissues of the brain.

Different types of magnetic coils are used for TMS. A figure-of-eight coil generates an electric field (EF) suitable for focal stimulation of the cerebral cortex. Induced currents flowing at the intersection of the coil loops produce a peak of current density in the brain that is several times higher under the intersection than around it [114]. The full potential of focal TMS was achieved with the introduction of navigated TMS (nTMS) [115], also known as navigated brain stimulation (NBS) [11], [116], [117]. The individual brain anatomy is visualized in an MRI-based head model and the relative position of the TMS coil can be tracked with respect to the head of the participant in real time. The paramount of nTMS is EF-based navigation, which enables an estimation of the location of the EF maximum in the brain and accurate stimulation of a selected anatomical target (Figure 2).

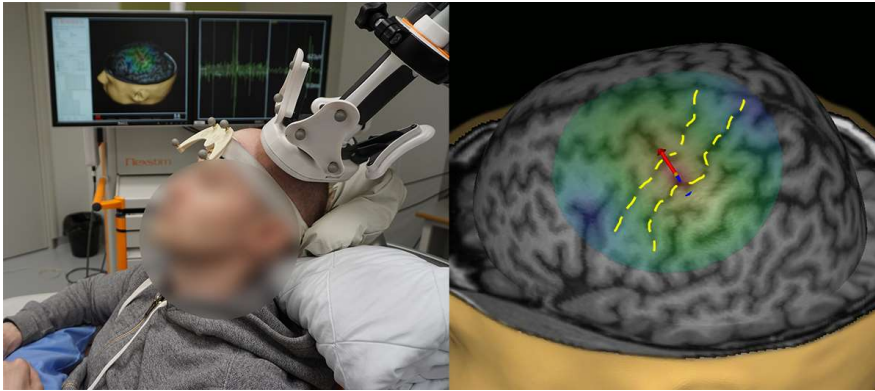


Figure 2 Electric field (EF) navigated transcranial magnetic stimulation. Left – a participant with a stimulation coil over his head. Right – a participant's 3-D head model with overlaid EF (green area) and EF direction (red and blue arrows). Stimulation is given to the abductor digiti minimi muscle representation located within the M1 area of the cerebral cortex (depicted with yellow lines). Photo published with permission of the participant.

The total accuracy (mean error of EF max estimation) of the modern nTMS system is several millimeters [11] and its sufficient for mapping of the motor cortex and reproducible stimulation.

The neural response to TMS is a complex interaction of physical (primarily properties of EF, amplitude, direction), anatomical (e.g. the direction of the targeted axons), and physiological factors (e.g. current level of excitability of the targeted neurons). TMS is thought to activate the superficial part of the motor cortex nearest to the scalp surface at the crown of the gyrus [118]. However, motor cortex TMS also activates neurons in the central sulcus [119] and distant sites of the brain [120].

The strength and depth of penetration of the induced electric field depend on the coil orientation [121]. When high TMS intensity is used, both direct activation of pyramidal cells at the bend of the axon in the border of grey and white matter in the sulcus and indirect transsynaptic activation of pyramidal cells via contacts from intracortical interneurons are possible. The mechanism of activation is modulation of neuron membrane potential by accumulated charges at axonal terminals or at their bends. An action potential is fired when membrane depolarization exceeds a threshold level. Thus, after a TMS pulse of sufficient intensity, multiple synchronous action potentials (direct D waves and indirect I-waves generated by cortical neural network) propagate in the corticospinal tract [11].

MEPs are electromyographic responses to TMS of a cortical muscle representation. They are recorded over the corresponding contralateral muscle using surface electrodes [122]. MEPs provide a general quantification of cortical and spinal excitability within selected corticospinal tracts [123],

[124]. Changes of MEP amplitude persisting after stimulation reflect durable changes in synaptic connectivity [102]. Thus, MEPs are useful in assessment of stimulation-induced changes and represent an independent source of information in addition to clinical examination in the evaluation of recovery [122], [125].

2.3.2 SPINAL MAGNETIC STIMULATION

Magnetic stimulation at the spinal level is a non-invasive method used as a painless alternative to percutaneous electrical stimulation [126], [127]. Spinal magnetic stimulation is performed routinely with a round coil over the cervical and lumbar spinal levels for peripheral motor conduction time (PMCT) measurement [128]. Brainstem magnetic stimulation can be employed for measurements of cortico-brainstem and brainstem-spinal root conduction times [126]. A double-cone coil is usually used for this purpose [129]. Stimulation outside the motor cortex provides information on the state of the corticomotoneuronal synapses, as responses elicited by the motor cortex stimulation are composite readouts of cortical and spinal excitability. Thus, development of spinal stimulation is useful for studies of induced neuroplasticity at the spinal level.

Determination of the coil location is currently based on external head landmarks in both spinal and brainstem stimulation [130]. The activation site can be rapidly shifted by slight dislocation of the coil [40]. In addition, accurate maintenance and retrieval of the coil position with low spatial variability during stimulation is challenging. The stimulations are consequently cumbersome and there is some uncertainty about the reproducibility of the results. Even the use of similar equipment cannot guarantee reproducibility of the studies. For example, Ugawa et al. [129] reported mean latencies of the responses to magnetic brainstem stimulation of 16.6 ± 0.7 ms recorded from slightly contracted first dorsal interosseous (FDI) muscle. Using the same type of stimulator, Martin et al. [130] reported mean response latencies from the same muscle of 18.1 ± 1.3 ms. However, the latency of the responses did not change when the muscle was contracted. Several different factors may be responsible for these inconsistencies.

After stimulation of cervical roots, MEP latency from resting FDI muscle varied from 13.2 ± 1.5 ms [128] to 14.6 ± 1.0 ms [130]. MEP latencies recorded from contracted FDI by another group [129] were relatively similar (12.7 - 13.0 ms) [128]. MEP latencies induced by cervical spinal stimulation from the abductor digiti minimi (ADM) have been reported to be 11.8 ± 1.0 ms [131], 12.7 ± 1.1 ms [131], 13.9 ± 1.8 ms [132], and 14.0 ± 1.5 ms [133]. The variability of MEP latency between the studies is approximately 3.5 ms for brainstem stimulation and approximately 4.7 ms for spinal stimulation. Moreover,

interindividual variability of the optimal stimulation site was found for brainstem stimulation [134] and can be easily anticipated for spinal stimulation. Such variability of latencies may indicate that both spinal and brainstem stimulation can potentially activate several distinct sites within the stimulated neural pathways and often the exact site of activation remains unknown.

2.3.3 PERIPHERAL NERVE STIMULATION, F- AND H-RESPONSES

Peripheral nerve stimulation (PNS) can non-invasively activate peripheral nerves with an applied EF [135]. The strongest and most selective stimulation with PNS is achieved when surface electrodes are in the proximity to a targeted nerve, restricting activation to a small portion of neural fibers. An increase of stimulation intensity may lead to a diffuse EF that decreases selectivity and produces unpleasant muscle twitches or sensations.

F-responses or F-waves are low-amplitude responses to PNS [136]. F-waves are elicited by supramaximal stimulation and are usually recorded from small hand or foot muscles innervated by the stimulated nerve. A PNS pulse generates APs propagating along the nerve from the distal site of activation in both orthodromic and antidromic directions [137]. Antidromic APs activate MNs and produce an F-response via their backfiring. F-waves are one of the most frequently used peripheral readouts of neural activity. F-response parameters include amplitude, minimum latency, and the percentage of detectable F-waves (persistence). The F-wave amplitude reflects activation of several motor units (MU) and latency indicates the conduction time between the distal site of activation and the corresponding MNs plus conduction time of backpropagation from MNs to a muscle where the F-wave is recorded. F-waves represent random activation of approximately 1% of MUs in the spinal motoneuron pool [138] and are composed of the corresponding MU action potentials (MUAPs). Therefore, all characteristics of F-waves are variable and a reliable analysis of F-waves requires recording of 10 to 20 F-waves.

F-responses provide information about axon and MU properties and the excitability of the postsynaptic part of corticospinal-motoneuronal synapses. F-wave amplitude and persistence did not change during strong voluntary contractions in a group of SCI individuals but increased in healthy control subjects [139]. MNs of partially paralyzed muscles after SCI may receive new inputs from nearby neurons; this can alter their excitability. Thus, changes of excitability may play a compensatory role. For example, mean F-wave amplitude at rest was larger in patients with SCI than in healthy controls [140]. F-waves were readily elicited in most chronic SCI patients with preserved CMAPs but only in half of patients with acute SCI [141]. F-wave minimum latencies tend to increase or remain unchanged in both acute and chronic SCI

[141]. F-response should not be confused with H-response, another late response which can be recorded from the muscle after stimulation of sensory fibers in the nerve. H-responses are useful in assessment of monosynaptic reflex activity in the spinal cord. Contamination of F-waves by H-responses can be avoided with supramaximal stimulation of the nerve [142].

2.4 PAIRED ASSOCIATIVE STIMULATION (PAS)

2.4.1 METHODOLOGY OF PAS

Paired Associative Stimulation (PAS) is non-invasive neuromodulation paradigm for experimental studies of neuroplasticity in healthy humans [143] and for neurorehabilitation after various disorders [55], [141], [142]. PAS is a dual stimulation consisting of TMS and PNS. One stimulus combination contains a TMS pulse applied to the cortical target and an electrical stimulus delivered to a corresponding contralateral peripheral nerve.

PAS is a stimulation of selected neural pathways and repeated pairing of TMS-PNS associations may increase or decrease their excitability depending on the TMS-PNS order and interstimulus interval (ISI) [143]. An excitatory PAS protocol was introduced in a seminal study by Stefan et al [12]. This was a prototype of PAS and is also called cortical PAS (Figure 3). A TMS pulse over the left hemisphere at the optimal site for activating the abductor pollicis brevis (APB) muscle was given after an electrical stimulus to the right median nerve. The protocol consisted of 90 TMS-PNS pairs with a constant ISI of 25 ms (PAS₂₅) and an interpair interval of 20 s. MEPs amplitudes increased right after the PAS. The effect persisted up to 60 min and was topographically specific. MEPs of other muscles changed only slightly or were not affected. The importance of TMS-PNS sequence and ISI was confirmed in the work by Wolters et al [144], where PNS of the median nerve preceded a single-pulse TMS by 10 ms (PAS₁₀) and led to MEP depression that persisted up to 90 min.

MEP facilitation was observed with ISI longer than 20 ms and ISIs of 0, 5, and 10 ms generated MEP inhibition [144]. However, robust MEP changes exceeding 20% in pre-post comparisons were obtained only with PAS₂₅ and PAS₁₀. Individualized approaches to estimate effective ISIs between TMS and PNS are based on e.g. I-waves [145], the latency of the N20 component of somatosensory evoked potentials (SEP) [146] or MEP and F-wave latencies [13]. These approaches can lead to more robust effects because individual conduction times are considered. This increases the probability of precise timing of pre- and post-synaptic spikes.

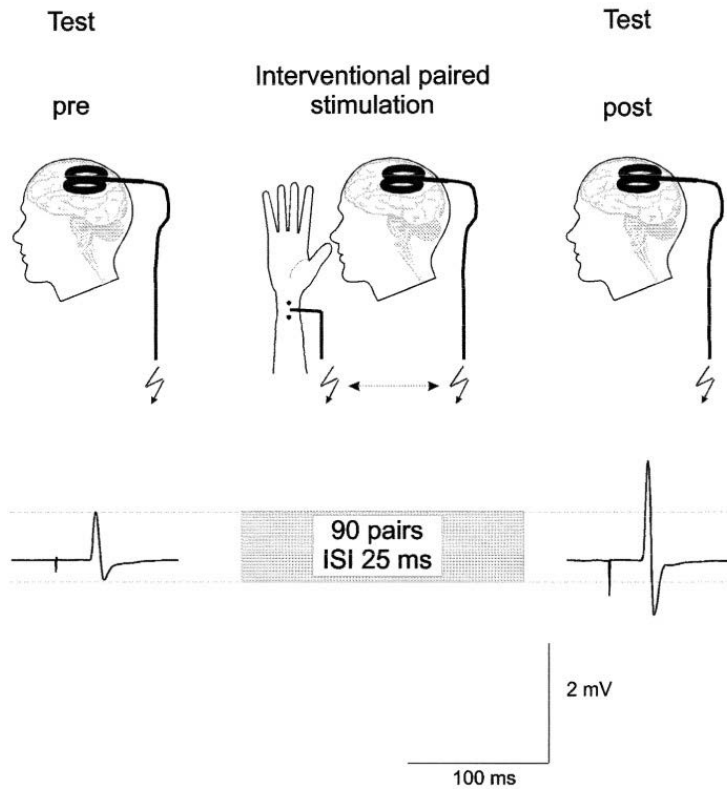


Figure 3 Experimental design of PAS25 excitatory protocol (top) and MEPs before and after stimulation (bottom). ISI – interstimulus interval. Figure reprinted from Stefan et al, 2000 with permission from Oxford University Press.

Thus, PAS-induced effects on neural excitability are rapidly evolving, reversible, persist beyond the period of stimulation, and are specific in terms of topography and neural pathways [143]. These PAS effects suggest durable induction of plasticity in targeted neural circuits.

2.4.2 NEURAL MECHANISMS

The theoretical background of PAS experimental design and interpretation of PAS-induced effects originates from studies of spike-timing dependent plasticity (STDP) [143], [147]. The cortical PAS protocol reproduces features of experimental design of the stimulation of cultured neurons [148] and neocortical slices [149]. Stefan et al [12] hypothesized that the ISI of 25 ms in PAS activates postsynaptic pyramidal cells synchronously, or shortly before

arrival of afferent signals from the hand S1 area via cortico-cortical connections. The temporal order of TMS and PNS was set to mimic the coincidence of synaptic inputs in cellular models of e.g. postsynaptic APs and unitary excitatory postsynaptic potentials (EPSPs) in dual whole-cell voltage recordings from pyramidal neurons [149]. When STDP is considered as a spike-based formulation of the Hebbian learning rule [150], PAS follows the logic of STDP studies performed in vitro [147]. Time-dependent activation of pre- and post-synaptic neurons can be reflected in PAS-induced activation of afferent pathways and cortical circuits within M1 [12]. Moreover, the PAS-induced increase of MEPs observed in numerous studies is consistent with the fundamental properties of STDP [143], [151]. Both PAS and STDP were found to work within a temporal window of a millisecond [143], [147]. Numerous studies have confirmed the effective range of ISIs (see [143] for review). The resemblance of PAS-induced changes to STDP was supported by the study [152] linking modification of PAS outcomes by pharmacological agents that target *N*-methyl-D-aspartate (NMDA) receptors, which are known to be a crucial component of STDP mechanisms [147], [149].

Limitations of linking STDP mechanisms to PAS, e.g. differences in complexity and modulation of effects by spontaneous firing rates and multiple synaptic inputs [143], [153], should be considered. These limitations can account for some contradictory data, e.g. absence of changes in short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), or short-latency afferent inhibition (SAI) after a facilitatory PAS protocol or decrease of SICI following an inhibitory protocol [143]. Many factors can potentially influence PAS outcomes and increase variability of the results. STDP models do not consider repetition frequency of the spike pairs [154] and other essential components, such as cascades of signaling events [147] back-propagating action potentials [155], voltage-dependent calcium channels [147], and other mechanisms. Models including several consecutive stimuli might improve understanding of STDP mechanisms [154] and consequently interpretation of PAS outcomes.

When repeated many times, PAS leads to long-lasting changes of excitability within the neural target [156]. The durability of PAS-induced effects is the most valuable property for clinical applications of PAS. Since the discovery of the stable increase of synaptic strength by tetanic stimulation in the hippocampus [157], the theoretical framework formulated by Hebb [150] has obtained experimental support from phenomena described as long-term potentiation (LTP) [147], [149], [151]. In PAS research, Hebb's postulate about the causal relationship between the repeated firing of the two neurons and corresponding durable increase or decrease of synaptic efficacy, specifically long-term potentiation (LTP) and long-term depressions (LTD) [158], were linked to PAS-induced potentiation and depression of MEPs [12], [159]–[161]. PAS protocols act at the system level and cannot selectively affect single

neurons. Thus, PAS-induced neuroplasticity represents an overall network response [162], including responses of different subnetworks and their interactions. The complex temporal pattern of PAS-induced effects can only partially be explained by results obtained in reduced cellular models [143].

2.4.3 PAS IN SCI REHABILITATION

Spinal PAS refers to protocols aiming to coincide TMS- and PNS-induced volleys at the synapses between UMNs and LMNs in the spinal cord to enhance corticospinal transmission. Augmentation of synaptic strength at the spinal level has clear therapeutic value for SCI, as it probably enhances motor control over paralyzed muscles. However, there is ambiguous evidence on whether cortical PAS alters excitability at the spinal level. Some reports support changes of excitability at the spinal level describing facilitation of the H-reflexes [163], [164], whereas other studies do not report differences in F-waves, suggesting absence of spinal changes [12], [144].

The hypothetical mechanism of spinal PAS is different from that proposed for cortical PAS and cortical PAS might not be able to induce similar effects [143]. UMNs are connected monosynaptically to LMNs innervating limb muscles [165]. TMS applied to the motor cortex evokes orthodromically propagating APs to the presynaptic terminals at the level of the spinal cord. PNS elicits antidromic volleys in LMNs that will travel along peripheral nerves up to the postsynaptic terminals in the ventral horns of the spinal cord and produce postsynaptic activation [166]. Thus, in contrast to cortical PAS, spinal protocols are designed to work purely via the corticospinal tract. However, concomitant activation of ascending pathways and other possible factors should also be considered.

The first spinal PAS protocol was established by Taylor and Martin [167]. In addition to traditional fixed ISIs, ISIs based on MEP latencies, maximum compound muscle action potentials (CMAPs), and responses to TMS of the cervical nerve roots were also used. The authors were able to increase and decrease CMEPs amplitudes by means of spinal PAS and thus demonstrated PAS-induced LTP- and LTD-like plasticity in spinal circuits [167]. Spinal associative stimulation (SAS) is a form of spinal PAS [168], [169] which combines a single-pulse sub-threshold TMS with PNS. SAS induced facilitation of H-responses at ISIs of 10 to 20 ms (early phase) or 70 to 90 ms (late phase) in a heterogenous group of patients and in healthy controls [168]. It was suggested that the stimulation targets the spinal cord on the basis of conduction time rationale and decreases the presynaptic inhibition of neural terminals. Repeated pairing of TMS and PNS with the early-phase ISI was used in an SAS protocol to modulate spinal excitability [170]. SAS increased spinal

excitability measured by the H-reflex during and after the intervention and was superior to PNS alone.

A more robust way of ISI calculation that ensures coincidence of orthodromic and antidromic volleys at the spinal level has been developed. Leukel et al [171] applied a 1-ms TMS delay in a spinal PAS protocol and found that conditioned H-reflexes were increased after both cortical and cervico-medullary stimulation, supporting the hypothesis about plasticity induction within the spinal cord. TMS alone did not produce this effect. Bunday and Perez [139] tested a spinal PAS protocol with the ISI designed to deliver TMS-induced volleys to the presynaptic terminals of corticospinal neurons 1 to 2 ms before antidromic volleys reached postsynaptic terminals of alpha motor neurons. A spinal PAS protocol enhanced corticospinal transmission and hand voluntary motor output in SCI patients and healthy participants. Decreased voluntary motor output and electrophysiological parameters were obtained when the reverse order of volley arrival and sham stimulation were used. These results demonstrated that STDP of residual corticospinal-motoneuronal synapses provided a mechanism to improve motor function after SCI. However, in another study [172], multiple high-frequency spinal PAS protocols (TMS and PNS were intended to reach the corticospinal-motoneuron synapses simultaneously) were not able to induce neuroplasticity in a consistent manner.

The potential usefulness of PAS in rehabilitation after brain injury was already anticipated in the earliest reports [12]. After this time, PAS research was mainly driven by possibility of clinical use. PAS has been investigated as a potential therapeutic approach not only for SCI [54], [102] but also for stroke [173], major depressive disorder [174], epilepsy [175], Parkinson's disease [176], hand dystonia [177], Alzheimer's disease [178], multiple sclerosis [179], migraine [180], schizophrenia [181], autism, and Asperger's syndrome [182].

Research on PAS clinical applications is a new area and the full therapeutic potential of PAS has not yet been realized [183]. PAS has several advantages important for its use in SCI therapy. TMS and PNS stimulators are available in hospitals worldwide and building a PAS setup is easy. The PAS rationale for returning motor control after SCI is simple. Interstimulus intervals are adjusted to coincide ascending and descending volleys at corticospinal-motoneuronal residual synapses [145]. Spared synaptic connections thus play a major role in mechanisms of spinal PAS [125]. Consequently, people with incomplete SCI are the main target group for spinal PAS therapy.

Evidence on the clinical efficacy of PAS is still incomplete and results are variable, especially due to the diversity of PAS protocols. Real and sham PAS in individuals with SCI produced similar improvement of motor and sensory function [184]; spasticity was not changed [184]. PAS induced an increase of MEP amplitudes that decreased to baseline level after 50 to 120 min both in patients with SCI and healthy controls [185]. PAS combined with muscle

contraction is more effective than PAS administered at rest, indicating that muscle contraction during PAS enhances corticospinal transmission [186]. PAS enhanced MEPs up to 30 min in patients with SCI and healthy controls and also increased spontaneous EMG activity and ankle dorsiflexion force in both groups, suggesting functional relevance of induced plastic effects [187]. A PAS-induced significant increase of corticospinal excitability up to 30 min was observed in healthy subjects and in SCI patients with good motor recovery assessed by SCIM but not in SCI patients with poor functional recovery [184]. Combination of TMS with PNS of the homonymous nerve, in contrast to heteronymous pathways, transiently (20 min) facilitated MEP amplitudes but only in patients with incomplete SCI and mild injuries, which suggests the importance of preserved transmission along sensory tracts [188].

A novel PAS protocol suitable for use in rehabilitation after SCI was introduced by Shulga et al [13], [14]. The increase in the number of orthodromic volleys was achieved by increasing TMS intensity; high-intensity TMS pulses result in a high-frequency repetitive discharge of corticospinal neurons [124]. To increase the number of antidromic volleys, high-frequency trains of PNS were used. This protocol reliably enhanced corticospinal transmission at a wide range of ISIs, plausibly due to the increased number of pre- and postsynaptic volleys and, consequently, their interactions [14]. The authors hypothesized that when LTP-inducing and LTD-inducing timing interactions occur at the same time, LTP can override LTD [189] during PAS, similarly to the mechanism shown by Sjöström et al [189] in a cellular model. This is especially useful considering the challenging conditions for EMG recording in SCI individuals and the absence of motor responses in some patients. Some voluntary movements were returned to fully paralyzed muscles and improved movement ability was detected after 1 to 6 months of PAS in several chronic para- and tetraplegic SCI patients [54], [190]. The improvements were stable for at least 1 month after the last stimulation. Further development of the protocol revealed its superiority to PNS in its ability to improve hand function in tetraplegic individuals [190] and even higher efficacy when increased PNS frequency was employed [191]. Currently, the possibility to improve hand muscle strength by means of novel PAS was demonstrated in group of participants with incomplete chronic SCI of traumatic [190] and non-traumatic origin [192].

In summary, spinal PAS is a promising therapeutic approach to enhance transmission via residual synaptic connections in the injured corticospinal tract and recover motor control over paralyzed muscles after SCI. Most studies investigating the therapeutic potential of PAS have had a relatively short duration. Accordingly, long-term interventions are warranted, which can provide crucial information for the development of PAS protocols suitable for routine clinical use.

3 AIMS OF THE THESIS

The aim of the thesis was to explore the possible therapeutic effects of long-term PAS on hand and leg motor function in individuals with chronic incomplete SCI of traumatic origin. This thesis consists of two intervention studies (**Study I** and **Study II**) and a method development study (**Study III**).

To achieve the aim, the following sub goals were set:

1. to explore the therapeutic potential of long-term PAS by providing it until full recovery of hand muscle strength or until improvements cease (**Study I**)
2. to investigate the therapeutic effects of long-term PAS on leg muscle strength and walking (**Study II**)
3. to probe the applicability of electronic coil location control for magnetic stimulation at the cervical spinal level and to assess the reproducibility of stimulation-induced motor evoked potentials (MEPs) (**Study III**)

4 MATERIALS AND METHODS

4.1 PARTICIPANTS

Study I was a case study. The participant was a 46-year-old, right-handed previously healthy male with tetraplegia of traumatic origin (AIS B, neurological level C7, 5 years postinjury). **Study II** included five individuals (3 females, mean age 60, age range 48-70, AIS D, neurological level C1 or C5, >2 years postinjury, Table 1) with tetraplegia of traumatic origin. In **Study I** and **II**, the inclusion criteria were cervical incomplete SCI of traumatic origin and age 18 to 70 years. Exclusion criteria were contraindications for TMS or MRI. The conventional rehabilitation and medication of participants were not modified during the intervention or the follow-up. **Studies I** and **II** were registered at clinicaltrials.gov (NCT03459885). For **Study III**, nine healthy right-handed volunteers were recruited (3 females, mean age 32, age range 22-42). All participants in all studies did not have contraindications for TMS or MRI and provided written informed consent before the study. Ethical approval was granted by the Ethics Committee of the Hospital District of Helsinki and Uusimaa.

Table 1 Patient characteristics before the intervention (Study II)

Patient	Gender	Age	Neurological level	AIS	Time postinjury (years, months)	Lower limb MMT score Left/Right	Walking distance (m)/Time (s)
1	Female	62	C1	D	3, 2	30/38	80/266
2	Male	56	C5	D	3, 0	35/26	43/205
3	Female	70	C1	D	2, 10	18/14	Non-ambulatory
4	Male	48	C5	D	12, 2	26/8	38/117
5	Female	62	C5	D	8, 8	43/32	86/107

Walking distance (m)/Time (s) - the maximum distance that patients were able to walk without a break and without assistance of another person. The corresponding total time was measured.

4.2 PAS PROTOCOL AND INTERVENTIONS

Study I and **II** were interventional studies where PAS was administered 5 times per week during first 2 weeks and 3 times per week during the subsequent period. Parts of CSTs supplying the weak muscles (MMT score 0-3) were selected for stimulation. The participants were seated in a comfortable chair (Figure 4, left). One session consisted of PAS of 4-6 hotspot-nerve pairs given in pseudo-random order and lasted in total 1.5 to 3 hours (20 minutes per nerve).

For paired associative stimulation, nTMS (0,2 Hz, 100% MSO) of the selected hotspots was synchronized with the first pulse of the PNS train given to the corresponding contralateral nerve (Figure 4, right) [14] to coincide the induced neuronal volleys at the level of the cervical (**Study I**) or lumbar (**Study II**) spinal cord. PNS intensity was defined individually for each nerve using minimum intensity evoking a visually distinguishable F-response. Both TMS and PNS were triggered by Presentation software (Neurobehavioral Systems Inc., USA). A PAS treatment session of one hotspot-nerve pair consisted of 240 TMS-PNS associations. The interstimulus interval for each pair was individually calculated using the formula of F-response latency minus MEP latency to ensure simultaneous arrival of TMS-induced volley with the first pulse of PNS train at the spinal cord level [13].

In **Study I**, the participant was asked to imagine corresponding hand movement during PAS of the right radial nerve and all stimulated nerves in the left hand as in previous studies [54]. Stimulations of right median and ulnar nerve were coupled with muscle activations. In **Study II**, the participants were instructed to perform lower limb movements corresponding to the stimulated CST parts, e.g. plantarflex the ankle and slightly flex the knee during tibial nerve stimulation. EMLA ointment was used for local skin anesthesia if the participant had an uncomfortable PNS-induced sensation.

4.3 TRANSCRANIAL MAGNETIC STIMULATION

In **Studies I** to **III**, magnetic stimulations were performed with a magnetic stimulator (Nexstim Ltd., Finland) with a figure-of-eight coil (biphasic pulses, outer diameter 70 mm) guided by the Navigated Brain Stimulation (NBS) system (software version 4.3). Individual structural T1-weighted MRIs were obtained for each participant with a 3T Siemens Verio scanner (Siemens Healthcare, Germany). These were used for building individual 3-D head models. In **Study I** and **II**, motor cortical mapping was performed for identification of TMS hotspots, which are sites within the M1 area where stimulation most readily elicited MEPs from the muscle of interest.

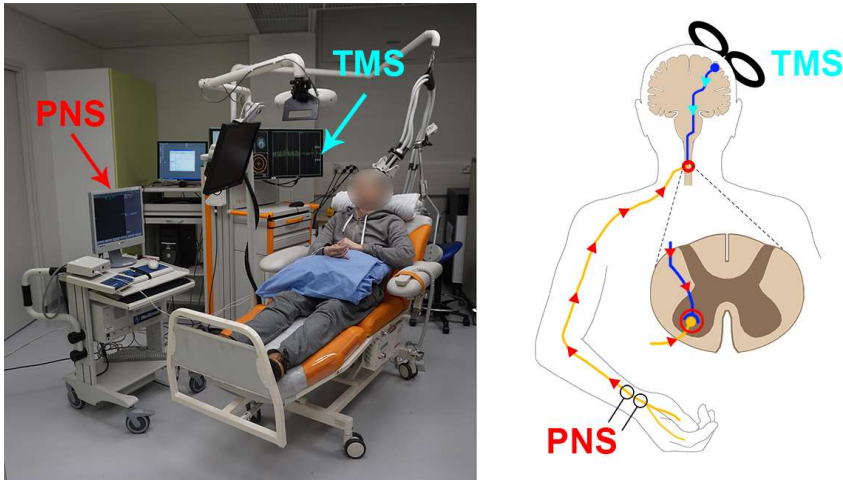


Figure 4 Left: PAS setup. Right: Basic principle of PAS. The interstimulus interval is adjusted to ensure arrival of arrival of the TMS-induced volley (blue arrows and blue line) with the first pulse of PNS train (red arrows and yellow line) at the level of corticomotoneuronal synapses in the spinal cord (red circle). Photo is published with permission from the participant. Figure is modified from Rodionov et al 2019 (Study I). The original publication is licensed under the Creative Commons Attribution 4.0 International License <https://creativecommons.org/licenses/by/4.0/>

In **Study I**, hotspots were found in both hemispheres for abductor pollicis brevis (APB, innervated by median nerve), abductor digiti minimi (ADM, innervated by ulnar nerve), and brachioradialis (BR, innervated by radial nerve) muscles (Figure 5, left). In **Study II**, hotspots were defined for abductor hallucis or soleus muscles (first and second choice, respectively, innervated by tibial nerve), tibialis anterior or extensor digitorum brevis muscles (peroneal nerve), vastus medialis or lateralis muscles (femoral nerve), and gluteus maximus (gluteal nerve) in one or both hemispheres (Figure 5, right). Recordings from the second-choice muscle were used if EMG recordings from the first-choice muscle were contaminated by spasticity-related muscle activity or MEPs were not detected.

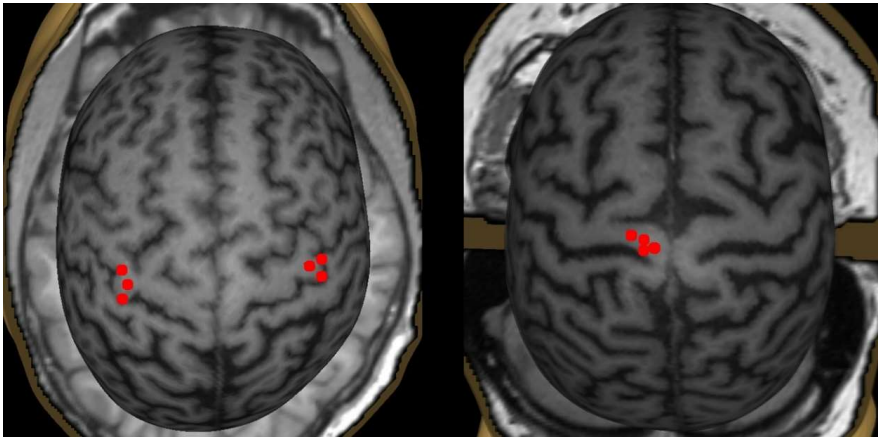


Figure 5 Typical stimulation targets for PAS of upper limbs (left) and lower limbs (right) presented with red points superimposed over individual 3-D head models of two different participants.

4.4 MAGNETIC STIMULATION AT THE CERVICAL SPINAL LEVEL

In **Study III**, magnetic stimulation was applied at the C2-C6 cervical spinal levels. During the search for optimal stimulation sites, the coil was placed vertically on the right part of the upper neck with its center approximately at the C1-C2 level (Figure 6, left). The stimulation intensity was 50% of maximum stimulator output (MSO). The coil was moved in steps of approximately 1 cm down to the C5-C6 level. At each step, 1 to 5 magnetic pulses were given. The coil was also turned by 15 to 30 degrees in both directions to find the position where a MEP with amplitude $>10 \mu\text{V}$ could be elicited.

The stimulation intensity was increased by 5% of MSO if no MEPs were obtained and the procedure was then repeated. Three MEP-producing coil positions were found in each subject prior to the experiment. Coordinates of the coil center and estimated electric field maximum (eEFM) were recorded. The appearance threshold (AT) of MEPs was measured. AT was defined as the intensity at which three consecutive stimuli could elicit a MEP with amplitude $\geq 10 \mu\text{V}$ each and stimulation at intensity 1% lower did not elicit any response. During the experiment, an intensity of 120% of AT was used to elicit 10 MEPs. Individual MEP peak-to-peak amplitudes and onset latencies were measured. The effect of coil position shifts on MEPs was probed by relocating or rotating the coil in 1- to 2-mm steps until a change in MEP amplitude exceeded 90% of the initial response. Corresponding coordinates were obtained.

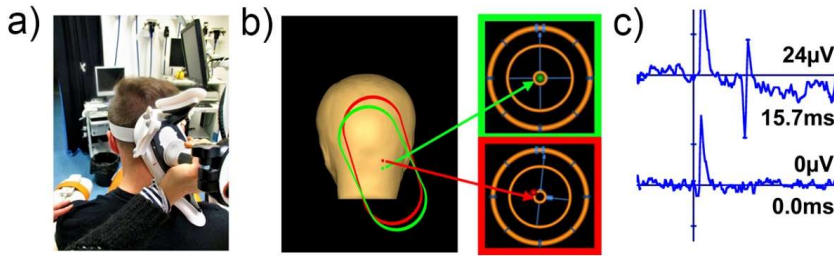


Figure 6 Location-controlled magnetic stimulation over the cervical spinal level. From left to right: a) the coil is re-positioned, b) targeted coil position (green), altered coil position (red), c) corresponding EMG traces obtained during targeted and non-targeted stimulation. Figure is modified from Rodionov et al 2019 (Study III). The original publication is licensed under the Creative Commons Attribution 4.0 International License <https://creativecommons.org/licenses/by-nc-nd/4.0/>

Input-output properties of the stimulated neural structures were explored by stimulating at the C7 level and cervical levels above it (C2 and C4) in three representative subjects. During stimulation, the intensity of magnetic stimulation was increased in 3% MSO steps from approximately 5% below AT to 140% of AT (at C7 level) or 90% to 100% of MSO (C2 and C4 levels).

4.5 PERIPHERAL NERVE STIMULATION

In **Study I** and **II**, PNS was delivered using a Dantec Keypoint electroneuromyography device (Natus Medical Inc., USA) and surface electrodes (Neuroline 720; Ambu A/S, Denmark, Figure 4). PNS stimuli were 100-Hz trains (1-ms square pulses, 6 pulses per train, train duration 100 ms). These stimuli were shown to be more efficient for MEP potentiation in healthy people [191]. Previously, 50-Hz trains (duration 50 ms) were used in part of **Study I** (before week 30). In **Study I**, PNS was delivered to the median and the ulnar nerves at the wrist and to the radial nerve at the radial groove (Figure 7, upper panel). In **Study II**, the gluteal nerve, the femoral nerve, the tibial nerve, and the peroneal nerve were stimulated (Figure 7, lower panel). For gluteal nerve stimulation, the electrode placement was determined by an anatomical landmark centered at the ischial tuberosity [193]. Crossing of the inguinal crease and femoral artery was selected for the femoral nerve stimulation [194]. The tibial nerve was stimulated behind the medial malleolus. The peroneal nerve was stimulated at the ankle.

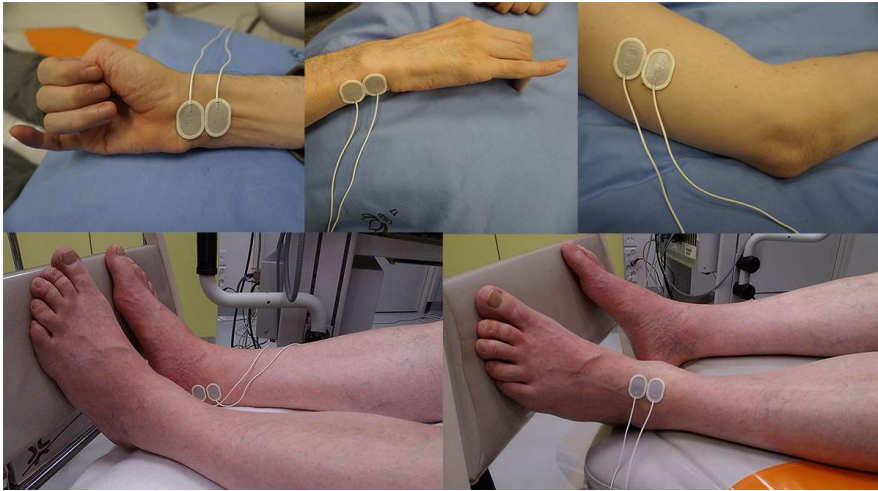


Figure 7 Electrode placement for PNS of (upper panel from left to right) median nerve, ulnar nerve, radial nerve and (lower panel from left to right) tibial nerve, peroneal nerve. Photos are published with permission from the participants.

4.6 CLINICAL EVALUATIONS AND FUNCTIONAL TESTS

Assessment of muscle strength was performed by an experienced physiotherapist with specialization in SCI. In **Studies I** and **II**, the Manual Muscle Test (MMT) [195] and in **Study II** the MMT and the lower extremity motor score (LEMS) of the standard AIS [50] were evaluated. In **Study I** and **II**, the same physiotherapist evaluated spasticity by employing the Modified Ashworth Scale (MAS) [196]. The participants' sensory function was assessed by a physician utilizing the AIS sensory score [50]. The participants' functional independence in **Study I** and **II** was assessed by the Spinal Cord Independence Measure (SCIM) [197]. In **Study I**, grip strength with the Jamar dynamometer, pinch strength with the pinch gauge [198], gross manual dexterity with the Box and Block (BB) Test [199], and fine manual dexterity with the Nine Hole Peg Test (NHPT) [200] were measured. In **Study II**, the maximum distance that patients were able to walk without assistance and the corresponding time were measured. See the corresponding sections of **Studies I** and **II** for more detailed information.

4.7 NEUROPHYSIOLOGICAL MEASUREMENTS

In **Studies I to III**, MEPs were recorded using the EMG device of the TMS system (band-pass filter 10-500 Hz, sampling rate 3 kHz, Nexstim, Finland) and the surface electrodes (Neuroline 720; Ambu A/S, Denmark) in a belly-tendon montage. In **Studies I and II**, MEPs were measured prior to the study to define ISIs of TMS-PNS pairs utilized in PAS protocol. The corresponding hotspots were used as stimulation targets. In **Study I**, MEPs were also collected during the intervention and in the follow-up. EMG signals were amplified, filtered, digitized, and stored in the NBS computer for offline analysis.

In **Studies I and II**, measurements of F-responses were obtained with a Dantec Keypoint electroneuromyography device (sampled at 48 kHz, band-pass filter 10-10 000 Hz) prior to the study to define ISIs of TMS-PNS pairs utilized in PAS protocol. Minimum latency (F min) and minimum intensity evoking a visually distinguishable F-response were determined from 10 F-waves. In **Study I**, mean amplitude and persistence were also measured before the intervention and in follow up.

4.8 DATA PROCESSING

In **Studies I and II**, the mean MMT score of all evaluated muscles and the mean MMT scores of the muscles innervated by each of the stimulated nerves (partial MMT) were calculated in each participant for each evaluation separately. The muscles with a value of 5 before the intervention were excluded from the analysis because no further improvement in these muscles could be observed. Walking speed was measured in **Study II**. The sum of points obtained with the MAS, AIS sensory scores, and SCIM was calculated for each evaluation separately. In **Studies I and II**, MEPs used for calculation of stimulation parameters in the PAS protocol were identified from 200-ms pre-stimulus and 100 post-stimulus intervals visualized with the EMG view of the NBS system. In **Studies I and III**, EMG signals were processed using Matlab. Individual peak-to-peak amplitudes and MEPs latencies were manually defined and corresponding mean values were calculated for each participant. In **Study III** the data were equally divided into two groups. Group 1 had eEFMs located above C1 in the head model (5 participants) and group 2 had eEFM below C1 (5 participants). Mean amplitude and latency values and mean MEP amplitude change between sessions for each day and group were calculated.

4.9 STATISTICAL ANALYSIS

In **Study II**, the Friedman test and Wilcoxon signed-rank test were used for comparisons of MMT, AIS motor scores, and walking speed. In addition, a binominal test was used for comparison of walking speed. Regression analysis was used to assess the relationship between the MMT score obtained before the PAS intervention and variation in the changes of walking speed during follow up. Model validation was performed with leave-one-out cross validation. Initial data set used for regression analysis was used to generate five training data sets each time omitting one of data points. Thus, first data set contained all data points except first one, second data set contained all data points except second and so on. Each training data set was used for building a regression model. Regression models were tested on corresponding omitted data points. Squared errors of estimates were recorded for each model. Average of squared errors was computed. Model validation was performed with leave-one-out cross validation. The initial data set used for regression analysis was used to generate five training data sets each time omitting one of data points. Thus, the first data set contained all data points except the first one, the second data set contained all data points except the second, and so on. Each training data set was used for building a regression model. Regression models were tested on the corresponding omitted data points. Squared errors of estimates were recorded for each model. The average of squared error was computed. In **Study III**, the reproducibility of MEP amplitudes was assessed using intra-class correlation coefficient (ICC) [201] on the basis of two-way analysis of variance (ANOVA). ICC=0.5 was used as the theoretical limit of reproducibility. Computations were performed with and without outliers. All statistical comparisons were performed in IBM SPSS Statistics (IBM Corp, Armonk, NY, USA, Version 24.0.). Results are reported as mean and standard deviation or mean and standard error of mean, quartiles, or median and range where appropriate.

5 EXPERIMENTAL STUDIES

5.1 STUDY I. RESTORATION OF HAND FUNCTION WITH LONG-TERM PAIRED ASSOCIATIVE STIMULATION AFTER CHRONIC INCOMPLETE TETRAPLEGIA: A CASE STUDY

5.1.1 BACKGROUND

The effect of one PAS session on corticospinal transmission persists beyond the period of stimulation. Multiple PAS sessions are thought to produce a more durable effect of larger magnitude. Thus, one of the key research questions in development of long-term PAS for rehabilitation after SCI is to what extent people with SCI can recover if PAS is provided for as long as improvement is observed. **Study I** investigated the gains in hand function in an individual with incomplete chronic tetraplegia. Long-term PAS was administered until full recovery of hand muscle strength occurred or until improvements reached a plateau.

5.1.2 STUDY DESIGN

In **Study I**, PAS was applied to both upper limbs (6 nerves in total) and lasted altogether 56 weeks (47 weeks of stimulation with 1-2-week breaks). The primary outcome measure was MMT. After 24 weeks of the intervention, PAS was combined with hand motor training of weak muscles in the right hand (MMT score 1 or 2 by the time training was started). The follow-up period continued for 32 weeks. Clinical evaluations and neurophysiological and functional tests were performed immediately prior to the study, during the intervention, and during follow-up.

5.1.3 RESULTS AND DISCUSSION

Study I revealed that the maximum hand MMT score corresponding to normal strength and range of motion of individual muscles can be restored by means of long-term PAS during the chronic stage, 5 years postinjury.

In both hands, motor control recovered from a non-functional state (no contraction in the muscles, no visible movement, or movement only with eliminated gravity corresponding to MMT scores of 0, 1, and 2) to a fully

functional state (Figure 8, a, b). The participant could hold the test position for 11 previously non-functional muscles against maximal resistance (MMT score 5) in the right hand and against moderate resistance (MMT score 4) for the remaining 4 tested muscles. In the left hand, the maximum MMT score was obtained in all tested muscles. The intervention consisted of 47 weeks of stimulation. Shorter exposure to PAS revealed effects of smaller magnitude [54], [190], justifying a longer and individually defined duration of PAS as a favorable treatment option.

The increase in MMT scores was accompanied by clinically meaningful and stable improvements in both hands, including remarkably increased grip and pinch strength and enhanced gross and fine dexterity (Figure 8, c, d, g, h). The outcomes were important for the participant because he regained the ability to perform complex motor tasks useful in everyday life. By the beginning of follow up, the participant could eat, bathe, dress, and groom independently and without adaptive devices. However, before the treatment he needed total or partial assistance for these tasks. Especially important was the return of the ability to write and press buttons and the alleviation of pain and subsequent decrease of pain medication (see **Study I** for more details).

The maximum MMT score in the left hand remained stable in the 32-week follow-up. At the same time, the right hand total MMT score further improved by 17 points, including changes from MMT score 1 to 4 (no visible movement in the muscle in holding the test position against moderate resistance) in some muscles. Regular 1- to 2-week breaks during the intervention did not hinder recovery. When PAS was combined with motor training (PAS-MT), pinch strength, which remained stable for many weeks in both hands during application of PAS alone, increased rapidly. In the follow-up, however, pinch strength returned to the level before PAS-MT administration. Repetitive practice is the primary therapeutic factor in many therapeutic approaches [202]. The combination of PAS with active physical rehabilitation can be advantageous because durable potentiation of the weak CST connections subsequently enables their engagement in signal transmission when stimulation is off. Thus, motor improvement can be sustained and even augmented e.g. by training or daily life activity.

Recovery of hand function was accompanied by an increase of MEP amplitudes in comparison with the corresponding values before PAS in five out of six muscles used for establishing TMS hotspots for the PAS protocol. MEP amplitudes remained increased for at least 16 weeks after PAS termination, indicating a robust durable enhancement of corticospinal transmission (Figure 8, e, f). This effect resembled the property of LTP-like plasticity [143], which persisted many months after stimulations were completed. The corticospinal origin of the effect is supported by absence of changes in AIS sensory scores, indicating that changes in sensation could not contribute to the observed MEP amplitude changes.

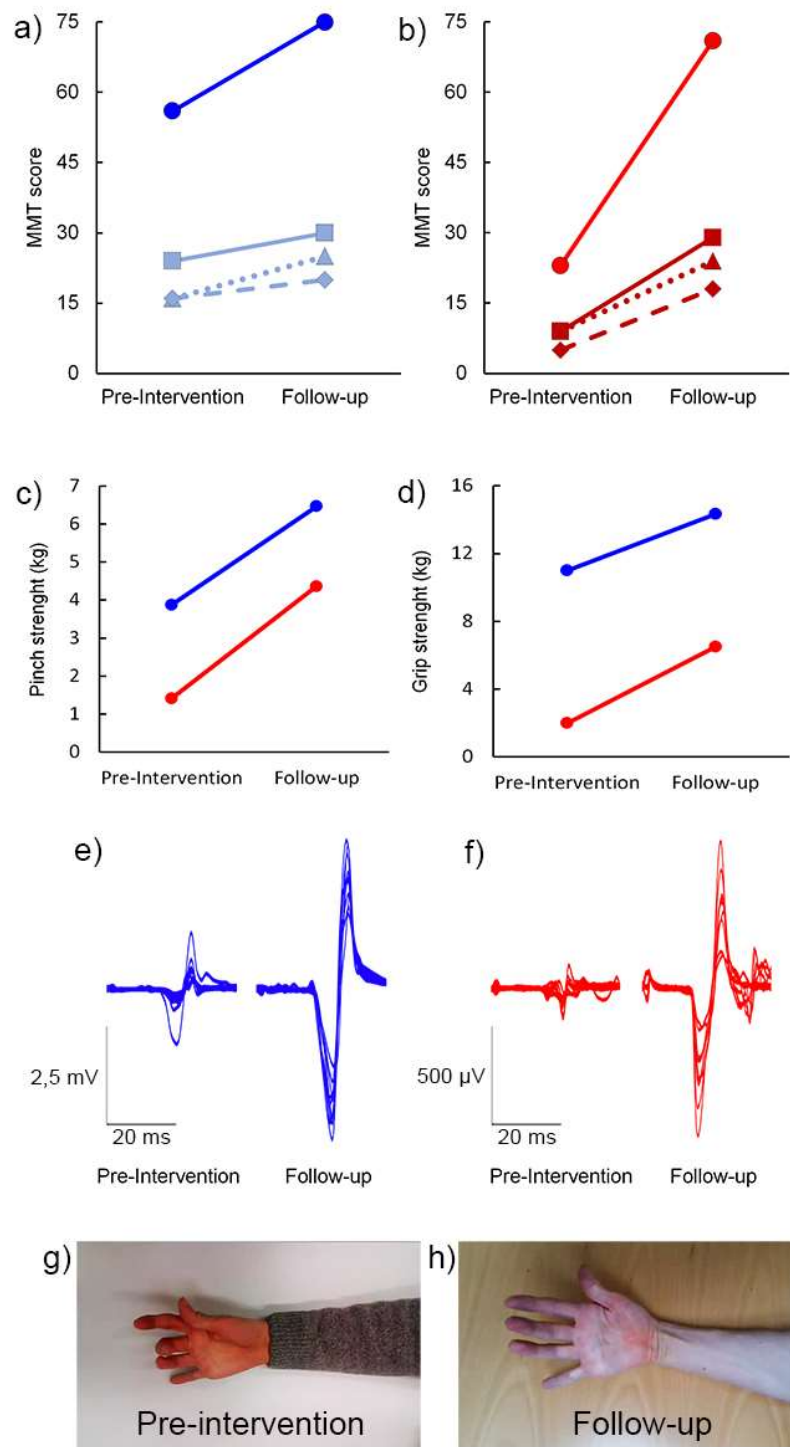


Figure 8 Manual muscle test (MMT) score collected before intervention and at the end of follow up in the left (a) and right (b) hand. Y-axis depicts the MMT score (the highest possible MMT score for all muscles evaluated is 75). Solid line and spheres depict MMT score of all evaluated muscles. Solid line and squares depict MMT score of the muscles innervated by median nerve; ulnar nerve values are represented by dotted line and triangles and radial nerve values by dashed line and diamonds. Changes in hand strength between pre-intervention and the end of the follow-up. c) The sum of tip, key, and palmar pinch tests results in the right (red) and left (blue) hand. d) Grip strength (red, right hand; blue, left hand). Y-axis represents weight in kg. Representative motor evoked potentials (MEPs) in the left (e) and right (f) hand. Changes in motor control: g) the participant could not fully open his palm before the intervention but h) could do it in follow up. Figure is modified from Rodionov et al, 2019 (Study I). The original publication is licensed under the Creative Commons Attribution 4.0 International License
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Changes of pathological continuous muscle contraction, or muscle stiffness are also unlikely as spasticity assessed with MAS was unchanged. However, growth of muscle bulk due to better innervation might influence MEPs. Overall MEP potentiation was slightly larger in the right than the left hand.

Individually defined long-term PAS administration is safe and tolerable. Over more than a year of regular stimulations, there was only one episode when a transient increase of spasticity and pain occurred. This episode coincided in time with infection and social stress experienced by the participant. After a 2-week break, the symptoms were alleviated and stimulations were resumed at the request of the participant. No other adverse effects were observed.

5.2 STUDY II. EFFECTS OF LONG-TERM PAIRED ASSOCIATIVE STIMULATION ON STRENGTH OF LEG MUSCLES AND WALKING IN CHRONIC TETRAPLEGIA: A PROOF-OF-CONCEPT PILOT STUDY

5.2.1 BACKGROUND

Locomotor function improvement after SCI remains clinically challenging. The high heterogeneity of the patient population [52], [69] and the pathologically reorganized CNS [84] and muscles [203] limit the effectiveness of existing treatments, particularly at the chronic stage. **Study II** explored the effects of long-term PAS on leg muscle strength and walking in a group of people with chronic incomplete tetraplegia.

5.2.2 STUDY DESIGN

In **Study II**, PAS was provided to the lower limbs of five individuals with SCI for 8 weeks, 5 times per week during the first 2 weeks (10 sessions) and 3 times per week during the subsequent intervention (18 sessions, Fig. 1). Clinical evaluations and walking test were performed immediately prior to the study, after 4 weeks of stimulation, after 8 weeks of stimulation, and after the 4-week follow up (without stimulation).

5.2.3 RESULTS AND DISCUSSION

Study II provided the first evidence that long-term PAS can increase leg muscle strength in a group of people with chronic tetraplegia (at least 3 years postinjury). A significant increase in total MMT scores of both legs was obtained by PAS (hereafter $n=5$ unless otherwise indicated, $\chi^2(3)=10.563$, $p=0.014$, Figure 9a). Total MMT scores were significantly elevated already after 4 weeks of PAS ($z=-2.023$, $p=0.043$) and remained significantly increased after 8 weeks of PAS ($z=-2.023$, $p=0.043$) and after a 4-week follow-up ($z=-2.023$, $p=0.043$) in comparison with the corresponding values before the intervention. The median (Md) increase of total MMT score right after the intervention was 1.23 (range 0.5-1.86) points in each evaluated muscle in each participant.

The effect was stable in the follow up (Md=1.15, range 0.42-1.86). AIS motor scores also increased significantly ($\chi^2(3)=8.733$, $p=0.033$, Fig 9b) by approximately 1 point per muscle. This was likely not due to spontaneous recovery, which usually plateaus by 12 months postinjury [7] with a minimal chance of further improvement [53]. A quantitatively similar increase of muscle strength by approximately 1 point was obtained in the previous studies [190], [192] where long-term PAS was applied for 1 month to improve hand motor function in a group of individuals with chronic incomplete tetraplegia. A longer intervention time in **Study II** could produce a stronger effect and could at least partially explain the comparability of the results of **Study II** and earlier studies despite differences in size of the leg and hand muscles. Leg muscles are larger and might require a longer intervention for obtaining a quantitatively similar improvement.

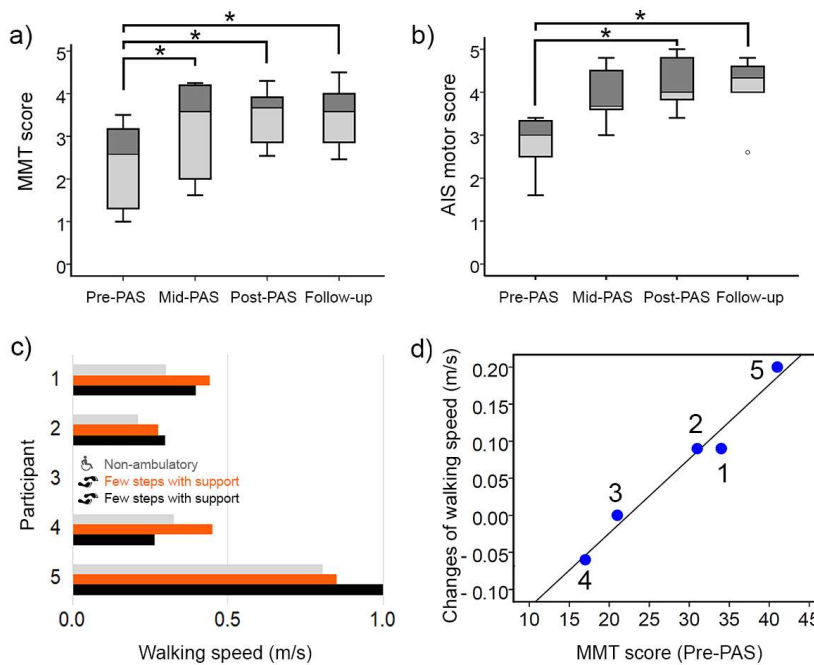


Figure 9 a) MMT scores and b) AIS motor scores before the intervention (Pre-PAS), after 4 weeks (Mid-PAS), and after 8 weeks (Post-PAS) of stimulations and in the 4-week follow-up. Asterisks show significant differences ($n=5$, $p<0.05$), c) walking speed before the intervention (grey bars), after 8 weeks (orange bars) of stimulations and in the 4-week follow-up (black bars), d) relationship between the sum of the MMT scores collected prior to the intervention (Pre-PAS) from the key muscles and changes in walking speed obtained in the follow up. Linear regression, solid line. Figure is modified from Rodionov et al, *in press* (Study II). The original publication is licensed under the Creative Commons Attribution 4.0 International License <https://creativecommons.org/licenses/by-nc-nd/4.0/>

In **Study II**, there were no significant differences between total and partial MMT scores. Changes of total and partial MMT scores did not differ after 4 weeks of PAS ($z=-0.135$, $p=0.893$), after 8 weeks of PAS ($z=-0.816$, $p=0.414$), or in the follow-up ($z=-0.365$, $p=0.715$). Thus, a similar increase was also obtained for partial MMT scores of the muscles innervated by stimulated nerves only. Improvements in all muscles, including those also innervated by non-stimulated nerves, could be due to more active use of the lower limbs during the intervention. However, this does not exclude other factors such as spread of activation to multiple sites within the M1 area or changes in the balance of excitatory and inhibitory circuits within spinal segments that supply corresponding peripheral nerves of the lower limbs. For example, high-

intensity PNS of the gluteal nerve can readily activate the sciatic nerve and high-intensity PNS of the femoral nerve might activate the obturator nerve. Walking speed did not change across all evaluations ($n=4$, $\chi^2(2)=3.500$, $p=0.174$). Nevertheless, walking speed significantly increased after 8 weeks of PAS (binomial test, $p=0.031$, Figure 9c). Qualitative improvement was observed in non-ambulatory participant 3, who was able to take several steps with Eva support walker after the intervention. In the follow up, participants 2 and 5 demonstrated an additional increase of walking speed, participant 3 remained stable, whereas walking speed decreased in participants 1 and 4. The walking speed remained increased in comparison with corresponding values before the intervention in all but one participant (participant 4, more than 12 years postinjury). MMT scores of key muscles, including gluteus maximus and gluteus medius, knee flexors, knee extensors, and ankle plantar flexors, predicted changes of walking speed after locomotor training. [204] Analogously, the MMT score of key muscles obtained before PAS intervention predicted well the changes in walking speed in follow-up ($R^2=0.962$). Cross validation revealed average error 0.001 ± 0.001 (mean \pm SD) which is negligible. Cross-validation revealed an average error of 0.001 ± 0.001 , which is negligible. The MMT score of key muscles in pre-PAS and changes of walking speed in the follow-up were linearly related (Figure 9d) and highly correlated ($r=0.975$, $p=0.005$). This can be particularly important for patient selection and estimation of the treatment duration. MMT scores of other muscles did not predict changes in walking speed. Participant 4 reported slightly increased spasticity and lower back pain which disappeared by the end of the intervention; no other side effects were observed.

5.3 STUDY III. THE USE OF ELECTRONIC COIL LOCATION CONTROL FOR FOCAL MAGNETIC STIMULATION AT CERVICAL LEVEL

5.3.1 BACKGROUND

In **Studies I** and **II**, TMS- and PNS-induced volleys elicited by long-term PAS were timed to coincide at the corticomotoneuronal synapses in the spinal cord for enhancement of corticospinal transmission. Magnetic spinal stimulation with the focal coil can be useful for probing excitability at different sites at the cervical spinal level. Neurophysiological readouts quantifying changes of spinal excitability are important for understanding of stimulation-induced changes. **Study III** aimed to develop a novel technique for accurate maintenance and retrieval of the focal coil position for spinal stimulation and probed neural excitability at the cervical spinal level.

5.3.2 STUDY DESIGN

In **Study III**, three consecutive stimulation sessions were performed on each of two different days. Three optimal stimulation sites were defined prior to the experiment in each participant. Each session consisted of 10 magnetic pulses given at three sites at the cervical spinal level and recording of corresponding MEPs. Estimated electric field maximum (eEFM) above the C1 cervical level (group 1) and below (group 2) was used to test the usefulness of the coil tracking system. Below the level of C1, movements of the neck and its shape compromise MRI-head co-registration and the accuracy of computations. Reproducibility of MEP amplitudes was assessed with intra-class correlation coefficient (ICC). The sensitivity of the location-controlled stimulation was probed by analyzing the MEP changes produced by small coil dislocations. The input-output characteristics of the stimulated sites were also explored.

5.3.3 RESULTS AND DISCUSSION

Study III demonstrated that the electronic coil location control can be useful for reproducible MEP measurements with cervical-level magnetic stimulation. In group 1, the ICC (3,1) across 2 days was 0.73. The corresponding value in group 2 was 0.59. This is the first use of the coil tracking system and an MRI-based model for accurate maintenance and retrieval of the focal coil position at the cervical spinal level. All participants were responsive to the stimulation and none of them reported about any adverse effects.

However, the eEFM cannot be used for estimation of the activation site in spinal structures. Computational models could provide such information. Magnetic stimulation at the cervical level mainly elicits small-amplitude responses (Figure 10). The median amplitude across 2 days was 23.2 μV (Range R=371.0 μV) in group 1 and 26.9 μV (R=374.2 μV) in group 2. Mean latency was 15.4 ± 0.1 ms in group 1 and 16.4 ± 0.1 ms in group 2. The latencies were shorter than the latencies of MEPs elicited by brainstem magnetic stimulation [40], [130], [134]. However, they also differ from latencies obtained after TMS stimulation of the spinal roots [205], [206].

Reproducible responses obtained with the proposed new method indicate that coil location control can assist in keeping constant the characteristics of the induced EF and, consequently, the site of excitation. The location-controlled stimulation was sensitive to even small shifts and rotations of the coil. These manipulations produced abrupt and substantial increase, decrease, or total disappearance of MEP amplitudes. The minimum difference of the coil center coordinates after shifts and rotations was 7.1 ± 9.5 mm.

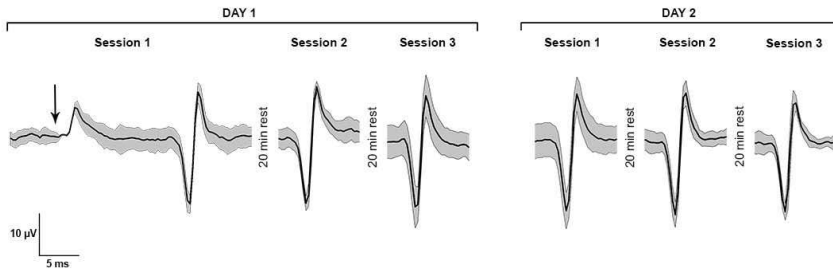


Figure 10 Averaged MEPs induced by magnetic stimulation at the cervical spinal level in representative participant 1. Grey areas represent 95% confidence intervals. The black arrow shows the stimulus onset followed by a unipolar stimulation artifact which is clipped off from EMG traces in subsequent sessions. Figure is modified from Rodionov et al, 2019 (Study III). The original publication is licensed under the Creative Commons Attribution 4.0 International License <https://creativecommons.org/licenses/by-nc-nd/4.0/>

The location-controlled focal magnetic stimulation can be useful in development of approaches for studies of specific corticospinal pathways.

Input-output characteristics were specific to each cervical level where stimulations were applied. At the C2-C4 levels, an initial increase of stimulation intensity did not affect MEP amplitudes until an individual threshold, defined for each participant, was exceeded. After the threshold, a steep increase of MEP amplitudes was observed except in participant 1, whose threshold was higher than the maximum stimulator output. A similar abrupt amplitude growth was obtained from stimulation at the C7 level close to the location of nerve roots supplying the ADM muscle. These results suggest multiple sites of excitation underlying the MEPs. Magnetic stimulation at the cervical level can potentially elicit intensity- and site-dependent activation at multiple locations, including motor roots and parts of peripheral nerves. Concentration of electric current can also occur within the intervertebral foramen as shown in some modeling experiments [127], probably indicating activation of the proximal segment of the spinal root. Thus, it is not possible to conclude that magnetic stimulation with the focal coil will exclusively activate only a single neural site as proposed in earlier reports [41]. Further research will benefit from the results of **Study III**, which enables reproducible MEP measurements in a test-retest study design. A focal figure-of-eight coil can be useful for a more directed form of stimulation and may be advantageous in the more specific stimulation of individual neural structures at the spinal level in comparison to a circular coil.

6 GENERAL DISCUSSION

6.1 CLINICAL VALUE OF LONG-TERM PAS FOR REHABILITATION AFTER SCI

Long-term PAS represents an example of successful ongoing translation of insights gained from neuroplasticity induction from experimental research [143] into promising clinical application improving voluntary motor control over paralyzed muscles [54], [190], [192].

A gap exists between experimental research on PAS and its implementation into clinical practice, largely because the number of studies investigating PAS-induced plasticity in spinal circuits is small. The majority of research is focused on changes of corticospinal excitability after a single stimulation session [143], [162], [207]. This greatly limits our understanding of long-term PAS efficacy. Durable changes in muscle strength and motor behavior remain generally unexplored. One of the most intriguing questions was to what extent the induced neuroplasticity within CST can be translated into stable clinically meaningful gains of motor function if multiple PAS sessions are applied. This thesis demonstrated that the strength of individual muscles can be restored from a non-functional to a fully functional state by means of long-term PAS. These improvements in individual muscles can lead to stable and clinically significant changes in whole-hand performance in daily activities. The results enrich existing research [54], [190], [192], where the same PAS protocol was employed for a shorter time. A comparative analysis with previous studies [54], [190], [192] suggests that the magnitude and stability of the overall PAS therapeutic effect can be positively related to the number of PAS sessions.

This thesis reveals the possibility of successful implementation of long-term PAS into current SCI rehabilitation with the ability to sustain and augment clinical effectiveness and address the patients' individual needs. Advances in early management of acute injury [6] have drastically increased survival rates after traumatic SCI [208]. This clinical population can potentially regain motor control at the chronic stage; however, this population is highly heterogenous and therefore requires individually tailored approaches. Residual corticospinal connections plausibly reinforced by long-term PAS [167], [183] are present in most cases [4], [5]. This greatly increases the number of people who can potentially benefit from the treatment. In **Studies I** and **II** and in other works employing the same protocol [54], [190], [192], improvements in muscle strength were observed in individuals with AIS B, C, and D and varying age, extent of preserved motor control, and postinjury time. This suggests that PAS may be useful for a wide range of patients.

Walking and most routine hand motor activities require bilateral limb movements. Although injuries to the spinal cord usually result in bilateral anatomical damage [209], [210], the extent of impaired motor function after SCI is often asymmetric in both upper [210] and lower extremities [211] and between different muscle groups [55]. PAS can be useful in ameliorating pathological asymmetry. The protocol is flexible and enables selection of the most promising cortical hotspot-nerve pairs for stimulation. For example, the weakest connections that might be difficult to reactivate by other approaches or the muscle groups with the greatest expected benefit for function can be targeted. Clinically effective PAS can be administered by prescribing an individual number of sessions and regulating their duration. In addition, limbs can be selected for stimulation according to individual therapeutic needs. When improvement in the selected muscle or muscle group is achieved, PAS of the CST supplying this muscle or muscle group can be discontinued.

Many basic functions of the motor system that are inactive after chronic SCI [212] can be reactivated. Moreover, the spinal cord possesses an intrinsic ability for adaptive reorganization [102], [213], which can be activated by external stimulation. Therefore, impairments can be gradually reversed. Selection of the optimal therapeutic target and administering effective PAS can lead to clinically meaningful rewiring of an injured spinal cord even at the chronic stage, many years after injury when recovery is rare [53]. Consequently, these neural changes can improve individual muscle strength and return of a wide range of motor activities. The results presented in this thesis support this view. This also suggests that a large therapeutic window exists for long-term PAS in chronic SCI. This may have important implications for comparison of cortical and spinal PAS in their ability to induce clinically relevant effects. It is possible that restoration of motor function by non-invasive stimulation after SCI depends to a large extent on its effectiveness in specific reactivation of the spinal cord rather than producing changes in cortical reorganization. Spinal PAS could be more effective for SCI. Long-term PAS represents a treatment modality that can create the optimal milieu for approaches aimed at retraining specific motor tasks and assembling them together. The latter represents the potential clinical value of long-term PAS for combinational strategies that are considered to be the most promising for maximizing recovery after SCI [69], [183], [214].

6.2 MECHANISMS OF LONG-TERM PAS

Despite differences in design between protocols, most studies on spinal PAS consistently reported an increase in spinal [167], [170], [171] or corticospinal excitability [54], [139] after PAS, implying involvement of LTP-like plasticity. From a long-term perspective, spinal PAS affects CST and the effect plausibly occurs at monosynaptic contacts between UMNs and LMNs in the spinal cord

[165]. This plasticity can be an initial response to stimulation underlying its therapeutic action.

The results presented in this thesis support a hypothesis that long-term PAS acts via accumulation of a single PAS effect that persists after stimulation. This effect is plausibly augmented by consecutive sessions. The increase of MEP amplitude obtained in **Study I** resembles properties of LTP-like plasticity but on a scale of many months, indicating that PAS-induced changes in the CST can be an important contributor to observed functional improvements. The quantitatively similar increase of individual muscle strength by approximately 1 MMT score that was observed in **Study II** and in previous research [190], where PAS was applied to CSTs supplying hand muscles, supports this view. In addition, **Studies I** and **II** and previous reports [54], [190], [192] did not reveal changes in spasticity and sensory function. When injury and synaptic connections plausibly strengthened by PAS are located at the same spinal level, it is difficult to conclude whether improvements after PAS are the result of modification of synaptic contacts between UMNs and LMNs or enhancement of transmission over the injury site. The results from **Study II** revealed that the injury location does not hinder the therapeutic effect. This again suggests involvement of corticospinal connections into the process of recovery.

However, the mechanism underlying long-term PAS therapeutic action appears to be more complex and probably cannot be fully explained by implications on synaptic plasticity derived from STDP models [189] or studies on single-session effects of PAS [14], [185]. Moreover, several different mechanisms acting within the CNS and at the level of MUs and muscles can be successively involved. This can be illustrated by the results of **Study I**. Changes in neurophysiological readouts were detected together with enhanced motor control over previously paralyzed muscles. However, functional improvements observed in the follow up occurred when stimulation was off, plausibly due to enhanced use of limbs in daily routines.

For further development of clinical application of long-term PAS, it is important to understand how PAS-induced changes within the injured CST are translated into gains in muscle force and how improvements in individual muscles are assembled into meaningful motor behaviors in individuals with SCI. MEP potentiation was observed in **Study I** already after several months of the intervention and MEPs remained increased in the follow up when compared with the values obtained before the intervention. However, the magnitude of this effect did not correlate with increases in muscle force. MEP amplitudes in the left hand were of similar size or larger than in the right hand before the intervention and despite their growth in both hands, interlimb MEP asymmetry remained approximately the same. However, the increase of MMT scores as well as grip and pinch strength in the right hand was clearly larger

than in the left hand. This implies the possibility of neural and neuromuscular mechanisms underlying long-term PAS therapeutic action.

Long-term PAS hypothetically can influence control over MUs. For example, recruitment of MUs, their firing rate, activation of fatigue-resistant MUs (which can remain intact many years postinjury [215]), or MU size can be enhanced. The size of the MUs can be increased in response to SCI [203] and stimulation could foster this process and promote sprouting of motor nerve terminals to more muscle fibers. These processes can potentially increase individual muscle strength. These neuromuscular mechanisms might require reactivation of descending input or work in parallel with it. These mechanisms may also lead to different effects in hand and leg muscles considering differences in the number and size of MUs between upper and lower limbs.

6.3 METHODOLOGICAL CONSIDERATIONS

The methodological basis of long-term PAS originates from the intersection of neuroscience, neurology, and neurophysiology. Thus, long-term PAS represents the result of interdisciplinary collaboration. Running long-term PAS as a clinical routine will require neurologists, clinical neurophysiologists, and physiotherapists and trained staff to administer PAS. PAS is currently a semi-automated procedure that requires manual setting of stimulation parameters and pressing PNS electrodes during stimulation and ensuring that patients comply with given instructions. Screening for contraindications to TMS and MRI (e.g. metal implants, implanted electronic devices, concomitant CNS disorders) is essential. Clinical evaluations of motor function are useful for planning individualized treatment and can even be employed for prediction of some functional outcomes, as indicated by the results of **Study II**.

Stimulation methods [126], [216], [217], neurophysiological measurements [122], [125], [185], [218], clinical inventory [49], [195], [196], functional tests [198], [200], and the self-reports [197] used in this thesis were shown to be reliable and can be recommended for use by researchers further developing PAS stimulation protocols and for health care professionals using long-term PAS as a novel treatment. The stimulation protocol was tested to induce LTP-like plasticity in a wide range of ISIs between TMS and PNS [13], [14]. It is feasible in challenging clinical settings. The rationale for the use of high-frequency PNS comes from in vitro studies [147], indicating that spike-timing relationships causing LTP could “win” over those producing an LTD effect in each pair of stimuli. The consensus that precise ISI is the main factor defining the direction of PAS-induced effects is currently challenged by research applying trains of stimuli [143], including the results of this thesis. An increasing body of evidence [147] indicates that when high-frequency bursts

are employed for stimulation, they can be considered as a basic element of synaptic modification but not individual spikes within the bursts.

Optimization of neuroplasticity and use of other factors that enhance corticospinal excitability changes induced by PAS [207], [219] are important methodological aims. Although combining PAS with motor tasks or motor imagery may be advantageous in generating additional excitability of corticospinal projections to stimulated muscles, this requires careful planning, especially regarding intensity and timing of these events [143]. The combination of PAS with intensive hand motor training in **Study I** additionally improved muscle strength. However, the increase in muscle force after MT was not stable during follow up and muscle strength values returned to the level before initiation of motor training. Protocols acting putatively via reactivation of spinal networks might not necessarily require simultaneous task-specific inputs [220]. Task-specific training aimed at improving voluntary motor control will require preserved connections within the spinal cord.

6.4 ADVANTAGES AND LIMITATIONS OF LONG-TERM PAS

Dual stimulation may be superior to single-stimulation modalities in improving motor control as shown in direct comparisons of paired stimulation and PNS [170], [190]. Long-term spinal PAS is a non-invasive approach to reactivate an injured spinal cord and uses the natural capacity of the CNS to reorganize. It works without surgery or implanted devices. All necessary equipment is usually available in large hospitals in developed countries worldwide. The stimulation protocol is feasible and was well tolerated by the participants. PAS can be administered as long-term treatment with low risk of adverse effects, even if the duration of the intervention exceeds a year (as demonstrated by **Study I**). If side effects emerge, stimulations can be temporarily discontinued. Increased spasticity or pain can be managed. The achieved therapeutic effects are not lost after stimulation pauses of 1 to 2 weeks. However, long-term PAS is a resource-intensive procedure that requires significant time for achieving therapeutic effects and commitment and cooperation from the patients. Although PNS can cause unpleasant sensations, these can be alleviated by application of local anesthetic or gradual increase of stimulation intensity. These factors should be carefully considered prior to the treatment.

Neurophysiological readouts such as MEPs and F-responses cannot always be obtained in individuals with SCI. Although spasticity-related artifacts contaminating EMG recordings and high RMTs can make motor mapping with TMS more challenging than in healthy subjects, this does not hamper application of PAS. In contrast, various contraindications to TMS or MRI and

the presence of brain injuries and psychiatric disorders can be exclusion criteria. Another limitation is a poor understanding of the causal relations between induced changes of synaptic transmission and indexes of functional recovery. The number of factors that influence the magnitude and direction of PAS effects makes prediction of PAS outcomes challenging, especially from a long-term perspective. Currently, measurement of PAS-induced plasticity is performed by comparison of MEPs obtained at different timepoints during PAS. Numerous external and internal factors can limit the practical usefulness of these data for prediction of therapeutic effects and monitoring of recovery.

This thesis describes the initial exploration steps of long-term PAS therapeutic effects in people with chronic traumatic SCI. **Study I** was a case study presenting results from only one patient. **Study II** also had a small sample size, but the statistically significant results provide a background for generalization of the findings in future research. A larger group of patients and a long individually planned duration of the intervention are required to provide further evidence on the efficacy of long-term PAS. Thus, the present results should be considered as exploratory. Another limitation of **Study II** was the absence of a control group. However, participants can be considered as their own controls because spontaneous recovery plateaus after 12 months [7] and the probability of spontaneous recovery at the chronic stage is low [53]. Taken together, the results of **Studies I** and **II** in combination with previous studies [54], [190], [192] applying the same stimulation protocol exclude the possibility of stochastically observed changes or spontaneous recovery underlying the observed functional improvements.

6.5 FUTURE PROSPECTS

Future research should focus on further investigation of the clinical efficacy of long-term PAS. Larger patient groups and administration of long-term PAS during the acute and sub-acute stages when pathological reorganization is active may provide new information on possible therapeutic effects. Automatization of the procedure is one of the possible short-term goals that may make PAS administration easier. A special focus should be decreasing the duration of the treatment.

Progress in non-invasive brain stimulation [11], [221] and a better understanding of the mechanisms of stimulation-induced plasticity [158], [162], [189] have made reactivation of residual spinal circuits by external stimulation a rapidly evolving trend in SCI rehabilitation [213]. However, several reviews [143], [162], [222] have highlighted the considerable interindividual variability in response to PAS, which can be influenced by many factors [143], [186] and most probably reflects their complex interactions [162]. The variability of the effects produced by spinal PAS remains generally unexplored. One potential future direction is

standardization of existing protocols and development of more efficient stimulation that would consider variability and produce quickly evolving and more stable changes of corticospinal excitability. The brain state and state of spinal circuits can significantly influence PAS results and should be controlled by increasing the number of experimental conditions, subjects, or trials. A possible approach is to provide stimulation only when a certain brain rhythm is detected in ongoing EEG [35] or the use of closed-loop stimulation [37] based on analysis of microstates of neuronal networks. Induced changes in the regions neighboring the targeted muscle representation should also be monitored.

Programmed cellular regrowth [223] is important for more effective recovery of injured CST. However, this remains a long-term goal mainly due to limitations of translation of experimental *in vitro* and animal studies into applications suitable for clinical practice [74]. No drug is currently proven to effectively trigger axon regeneration [224]. A combination of PAS with already available pharmacologic agents [70]–[72] appears to be a challenging but potentially fruitful direction, e.g. in testing the possibility to facilitate reactivation of neuronal circuits by this combinatory approach.

Basic research and computationally explicit models are needed for a better understanding of the mechanisms of PAS therapeutic action and for prediction of long-term PAS outcomes. Studies that provide a direct comparison of the effects of cortical to spinal PAS protocols would assist in their validation. Both neurophysiological and functional variables should be analyzed to form a synthesis that appears to be a reliable starting point for prediction of long-term PAS clinical outcomes. Some preliminary data may guide future research. One of the most promising approaches is the correlation of MEPs with indexes of functional recovery [94]. The presence of MEPs was observed in 70% of acute SCI patients who recovered walking ability and MEP latencies were normal in 80% of patients who achieved full walking capacity [107]. However, the correlation of MEPs and recovery seems to be nonlinear and complex.

The effectiveness of long-term PAS will benefit from early assessment and continuous monitoring of induced neuroplastic effects at the spinal level. The results of clinical evaluation of motor function were useful for planning individual treatments and prediction of outcomes as indicated by the results of **Studies I** and **II**. However, these results do not provide information on corticospinal conductivity and, most importantly, information on the intrinsic ability of the CNS to reorganize in response to stimulation. Representative neurophysiological data may also make it possible to adjust stimulation parameters according to the magnitude of PAS response at a very early phase of the treatment when clinical evaluation methods might be not sensitive enough to detect the induced changes.

Neurophysiological parameters quantifying changes of excitability within the spinal segment consisting of synaptic connections between UMNs and

LMNs can provide information on induced neuroplasticity. Responses to non-invasive spinal magnetic stimulation may be useful for this purpose. The availability of MRI-guided EF-navigated magnetic stimulation for accurate and reproducible selective activation of the CST, spinal roots, and nerves at the spinal level would be of great benefit. **Study III** revealed the usefulness of coil location control for selective stimulation of distinct sites at the spinal level. There is still no agreement whether axons of cortical MNs descending within lateral motor tracts can be activated by focal magnetic stimulation applied over the cervical spine [40], [126], [205], [225]. The spinal neurogeometry has a strong effect on the induced EF [226]. The sites where axons are bent will be easily activated because of their high excitability [227]. Moreover, neural excitability changes along the spinal cord. A much greater stimulation intensity is needed for activation of the cauda equina within the lumbar spine than the cervical roots [225]. Development of focal magnetic coils for spinal stimulation and modelling of induced electric fields during magnetic stimulation over the spine would predict how the EF spreads in complex patterns of individual anatomy and provide a background for new emerging technologies.

7 SUMMARY AND CONCLUSIONS

In this thesis, a spinal PAS protocol with a high frequency peripheral component was administered as a long-term experimental treatment in people with chronic incomplete SCI of traumatic origin. The aim was to explore its possible therapeutic effects in the upper and lower limbs. The obtained results provide new important information about the achievable therapeutic effect, improvements in hand and leg function, and several fruitful directions for further research on PAS efficacy. The thesis also introduced a new technique for the use of electronic coil location control for focal magnetic stimulation at the cervical spinal level. The results presented in this thesis and other works exploring the therapeutic effects of spinal PAS provide promising but preliminary evidence on the efficacy of long-term PAS for returning motor control after chronic SCI. Taken together, this work justifies further research on long-term PAS therapeutic efficacy, specifically, randomized double-blind sham-controlled clinical trials involving larger patient cohorts.

All the sub-goals of the thesis were achieved:

1. The maximum hand MMT score corresponding to normal strength and range of motion of individual muscles can be restored by means of long-term PAS, which leads to clinically meaningful, stable improvements of motor function (**Study I**)
2. Long-term PAS significantly increases leg MMT score, producing stable improvements in leg muscle strength (**Study II**)
3. Electronic coil location control is useful for reproducible MEP measurements with focal magnetic stimulation at the cervical spinal level and amplitudes of stimulation-induced MEPs are highly reproducible (**Study III**)

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